UNIVERSITY OF ALBERTA

PROTOZOAN BLOOD PARASITES OF RUFFED AND FRANKLIN'S GROUSE IN ALBERTA

BY

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A THESIS

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The undersigned certify that they have read and recommend to the Faculty of Graduate Studies for acceptance a thesis entitled "Protozoan Blood Parasites of Ruffed and Franklin's Grouse in Alberta" submitted by Martin Willard Newman in partial fulfillment of the requirements for the degree of Master of Science.

ABSTRACT

Blood smears and organs from 40 ruffed grouse collected in central Alberta between May and October 1966 were examined for the presence of blood protozoa. In addition, blood smears from 30 ruffed grouse and 34 Franklin's grouse, captured or shot in the foothills of southwestern Alberta, were examined.

Hematozoa of the genera Leucocytozoon, Haemoproteus, and Trypanosoma were found in blood smears of both species of grouse. The respective prevalence of these genera in central Alberta ruffed grouse was 60, 17, and 14%. In southwestern Alberta their prevalence in ruffed grouse was 52, 26, and 35%, and in Franklin's grouse, 76, 82, and 29%.

Asexual stages in the life cycle of Leucocytozoon were found in central Alberta ruffed grouse. Hepatic schizonts were seen in liver sections of 30 birds, and megaloschizonts were seen in kidney sections of two birds. These schizonts and possible histopathological changes in the organs are described.

Differences in hematozoon prevalence between the two species of grouse and between the two areas of Alberta are discussed with relation to the age of the bird, season of year, presence of possible vectors, and other epizootiological factors. Most apparent differences can be explained on the basis of the composition of the samples from the three populations; however, the high prevalence of Haemoproteus in Franklin's grouse appears to be a real though at present unexplainable difference. The data are compared with other studies of grouse hematozoa, and the possibility of mortalities being caused by these parasites is discussed. No definite conclusion concerning mortalities can be reached from the available data. However, with the finding of megaloschizonts of Leucocytozoon--the stage of the parasite's life cycle

which is associated with death in *Leucocytozoon* infections of Anseriformes--the possibility for parasite-induced mortalities cannot be dismissed.

Two appendices which list the possible dipteran vectors of grouse hematozoa found in Alberta and discuss the type of blood cells invaded by *Leucocytozoon* are included.

ACKNOWLEDGMENTS

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INTRODUCTION

Grouse have always been among the most popular game birds in northern Europe and North America. Periodic declines or "crashes" in populations of these birds have long given concern to sportsmen and biologists, many of whom believed that disease was responsible. Shortly after the turn of the century this concern led to the formation, in Great Britain, of the "Committee of Inquiry into Grouse Disease". The results of this commission's study were published in 1911 as The Grouse in Health and Disease, and dealt almost exclusively with the red grouse (Lagopus scoticus). Although these grouse were found to harbor many parasites and potential pathogens, the committee concluded that the cause of "Grouse Disease" was the presence of large numbers of Cobbald's Trichostrongylus in the caeca of adult grouse. Another disease, deemed to be of lesser importance, was coccidiosis suffered by immature grouse.

Subsequent studies of other grouse have failed to confirm these two parasitic conditions as being the causative agents in cyclic fluctuations of the numbers of grouse (Stafseth and Kotlan, 1925; Erickson, Highby and Carlson, 1949; Herman, 1963). Many other possible pathogens have been studied. In her compilation of references to the diseases of wild mammals and birds, Halloran (1955) lists 125 references to grouse, while Braun and Willers (1967) list 150 references to helminth and protozoan parasites of North American grouse. Many of these investigations involved the study of blood parasites.

Early studies of avian hematozoa

Ever since Laveran discovered the etiological agent of human malaria in 1880, biologists have been looking for parasites in the blood

of animals (trypanosomes had been reported previously from the blood of salmon and frogs, but these reports did not generate the intense interest in hematozoa that came about after Laveran's discovery of Plasmodium in humans). The first protozoan blood parasite to be described from birds was Polimitus major from the blood of the tawny owl (Syrnium aluco) and the long-eared owl (Asio otus) (Danilewsky, 1889). From the figures accompanying Danilewsky's second paper (1890) on avian blood parasites, it is evident that P. major is a leucocytozoon. A year after Danilewsky's description of Polimitus a second type of avian hematozoon came to light with description by Kruse (1890) of three species of Haemoproteus from the gray crow (Corvus cornix), the Spanish sparrow (Passer hispaniolensis), and the pigeon (Columbia livia). A third genus, Plasmodium, was described from the skylark (Alauda arvensis) by Labbé (1894).

The extensive study of avian hematozoa which was sparked by these early workers continued into the 20th century. Many new species were named, but in some cases the descriptions given were extremely brief and incomplete. This shallow approach to the study of these organisms has left much confusion in its wake. Coatney and Roudabush (1936) and Coatney (1936; 1937) in checklists of the genera Plasmodium, Haemoproteus, and Leucocytozoon have gathered together all of the early literature on these parasites. Coatney's catalog of the genus Leucocytozoon alone contains 68 species.

Studies of grouse hematozoa

The first grouse found to harbor protozoan blood parasites was the red grouse (Lagopus scoticus) (Seligmann and Sambon, 1907; Fantham, 1910a) in which were found Leucocytozoon lovati and Haemoproteus mansoni. These parasites were discovered as the result of the grouse disease

inquiry. The following year *L. mansoni* was described from the capercaillie (*Tetrao urogallus*) (Sambon, 1908). This hematozoon was again reported by Böing (1925). *Leucocytozoon*, *Haemoproteus*, and *Trypanosoma* were reported from the hazel hen (*Tetrastes bonasia*) by Oliger (1940) and from the hazel hen, capercaillie, and black grouse by Borg (1953).

In the 1930's North American investigators began looking at hematozoa from the native tetraonids. *Leucocytozoon*, *Trypanosoma*, and *Haemoproteus* were reported from the ruffed grouse (*Bonasa umbellus*) and the spruce grouse (*Canachites canadensis*) by Clarke (1935). The same year *Leucocytozoon* was reported from the willow ptarmigan (*Lagopus lagopus*) by Allen and Levine (1935) and from the sharp-tailed grouse (*Pedioecetes phasianellus*) by Saunders (1935). *Plasmodium* was found in sharp-tailed grouse by Wetmore (1939). Fowle (1946) reported *Leucocytozoon*, *Haemoproteus*, and *Trypanosoma* from the blue grouse (*Dendragapus obscurus*).

Purpose of the present study

The primary motivation for undertaking the present study was an interest in population fluctuations of grouse. In spite of the numerous studies of grouse blood parasites which have been made, little is understood of the relationship (if any) between hematozoon infections and population fluctuations (Herman, 1963). A second reason for undertaking this study was the lack of information on the hematozoa of grouse in the aspen-parkland habitat and Rocky Mountains region of Alberta, although some data from the latter region were presented by Holmes and Boag (1965). The virtually unknown nature of the asexual stages of grouse blood parasites provided a third topic of investigation. Finally, an attempt was made to elucidate the reliability of the blood-smear method of determining

incidence of parasitism by hematozoa. This technique is almost universally employed in surveys for hematozoa, and it was felt that by simultaneously making blood smears and sampling tissues a better indication of the incidence of hematozoa in a grouse population could be obtained.

As an aid to understanding the material presented in this study, hypothetical life cycles of the genera, Leucocytozoon and Haemoproteus, are diagrammed in Figures 1 and 2.

Figure 1. Life cycle of Leucocytozoon.

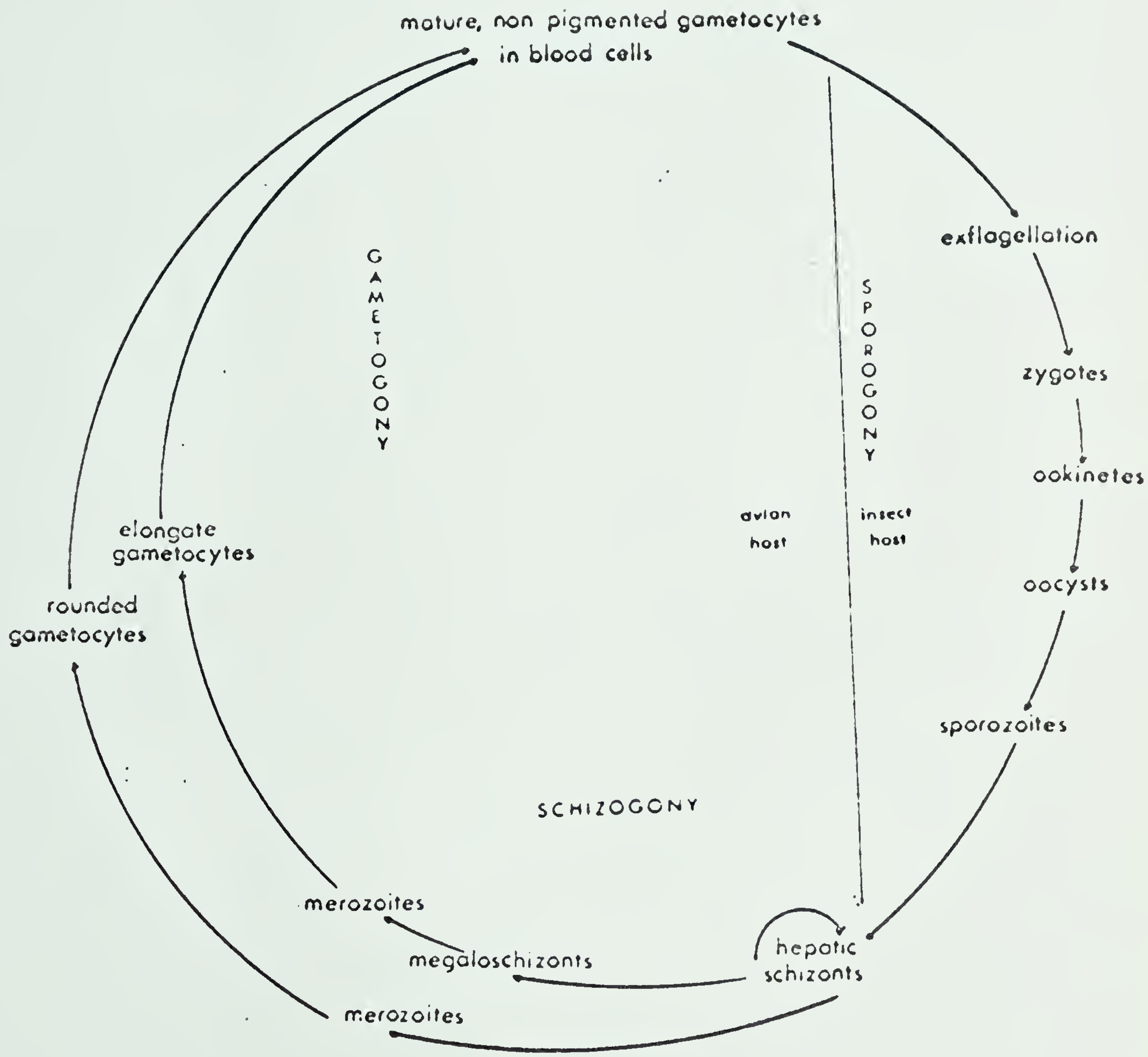
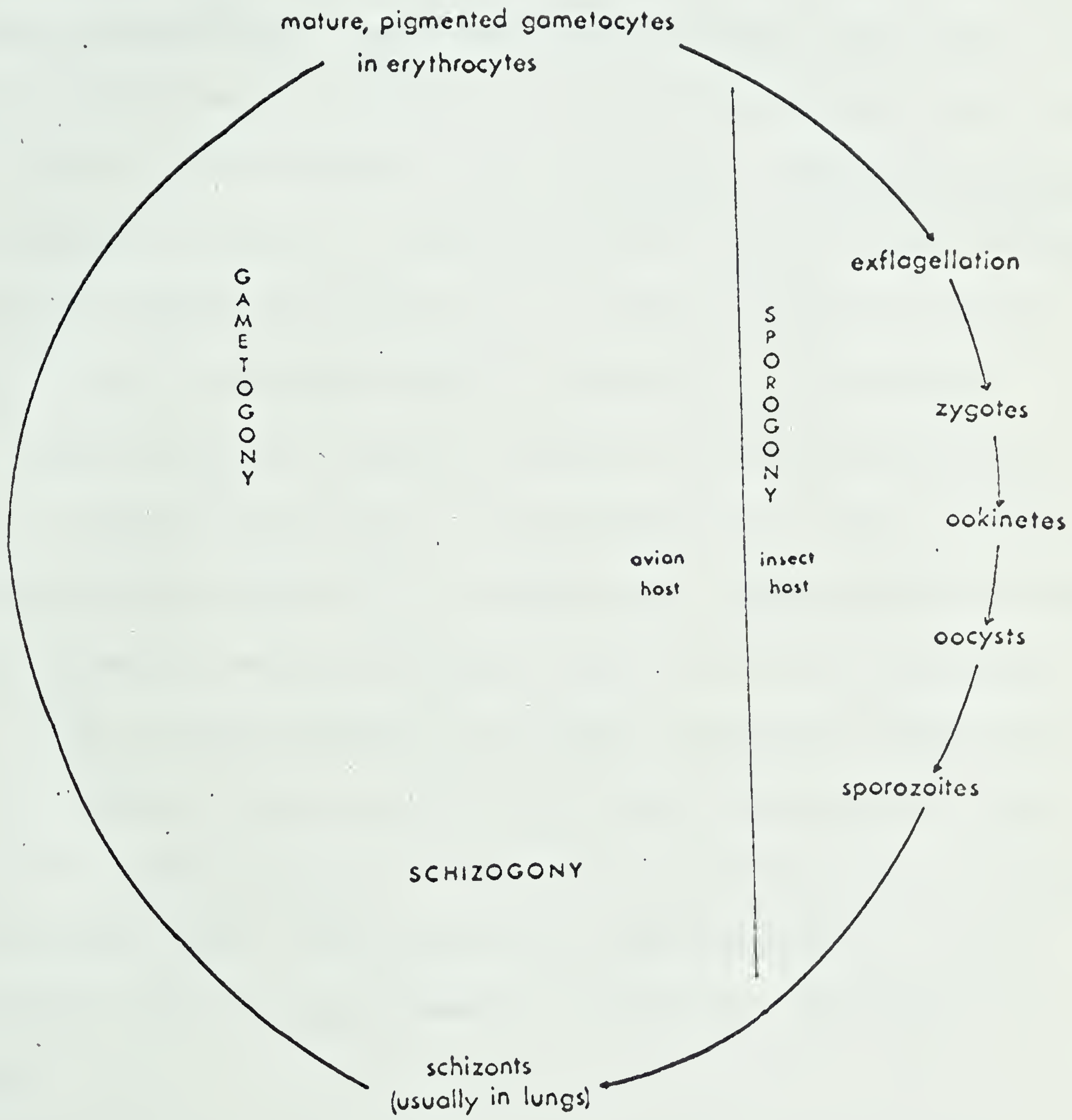

mature, non pigmented gametocytes
in blood cells
exflagellation
zygotes
ookinetes
oocysts
sporozoites
GAMETOGONY
SPOROGONY
avian host
insect host
elongate gametocytes
rounded gametocytes
SCHIZOGONY
merozoites
megaloschizonts
hepatic schizonts
merozoites

Figure 2. Life cycle of *Haemoproteus*.


mature, pigmented gametocytes
in erythrocytes
exflagellation
zygotes
ookinetes
oocysts
sporozoites
schizonts
(usually in lungs)
GAMETOGONY
SPOROGONY
SCHIZOGONY
avian
host
insect
host

MATERIALS AND METHODS

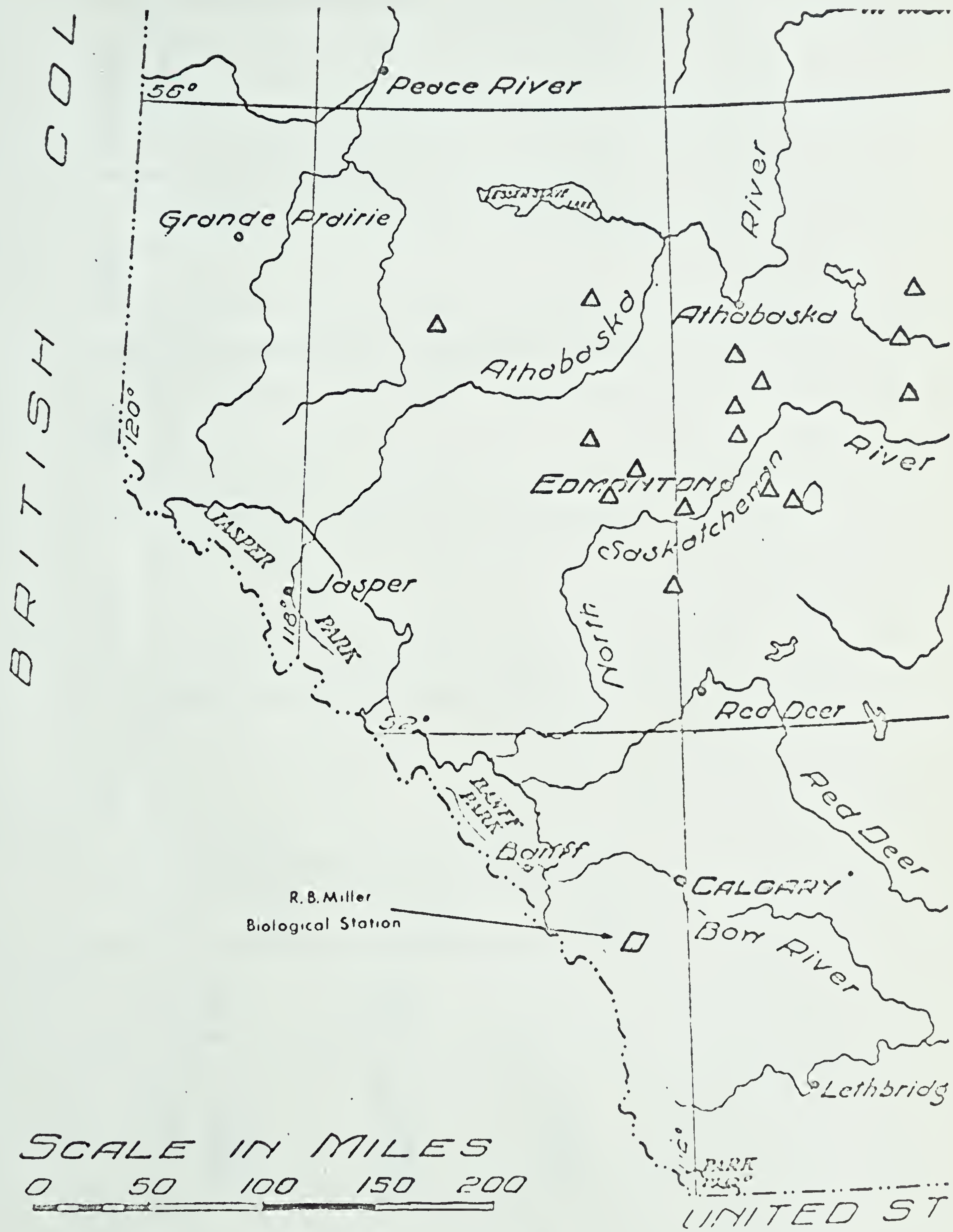
Field techniques

Blood smears and parts of organs were taken from ruffed grouse shot in central Alberta in 1966. Thirty-six of these grouse were collected by myself or in my presence. Four more were obtained at a provincial fish and game department hunter-check station. Figure 3 shows the sites where these birds were collected. Usable peripheral or heart blood smears were obtained from 35 of the above grouse. In addition, peripheral smears from 30 ruffed grouse and 34 Franklin's grouse (Canachites canadensis franklinii), made by colleagues at the R. B. Miller Biological Station in the foothills of southwestern Alberta (see Figure 3), were made available for use in this study. Table I shows the dates of collection of this material.

Peripheral blood of grouse collected in central Alberta was obtained by clipping a bird's toe, or occasionally from a head or neck wound when the former method did not yield blood. Because Franklin's and ruffed grouse handled near the Biological Station were to be banded and released, their blood was obtained by pricking the brachial vein with an insect pin. Clotting occurred rapidly after death and peripheral blood had to be taken within seconds if it was to be obtained at all. Erickson, Highby and Carlson (1949: 189) observed this same phenomenon -- "Dead grouse, we soon discovered, are almost as difficult to get blood out of as turnips."

Heart blood smears were made only from the central Alberta grouse. The heart was exposed as soon as possible after death. A small disposable syringe fitted with a 22-gauge needle was rinsed with a sodium citrate solution, introduced into a ventricle, and the blood was withdrawn.

Figure 3. Sites of collection of grouse blood and tissues.



BRITISH COL
56°
Peace River
Grande Prairie
River
Athabaska
Athabaska
River
120°
EDMONTON
Saskatchewan
North
JASPER
Jasper
118°
PARK
52°
Red Deer
Red Deer
BANFF PARK
Banff
CALGARY
R. B. Miller
Biological Station
Bow River
Lethbridg
SCALE IN MILES
0 50 100 150 200
UNITED ST

Table I. Numbers of blood smears taken from ruffed and Franklin's grouse over the period May to October.

Species	Location	Year	Age	May		June		July		August		September		October		Total
				1-15	16-31	1-15	16-30	1-15	16-31	1-15	16-31	1-15	16-30	1-15	16-31	
Ruffed grouse	Central Alberta	1966	Ad.	9	1	2	0	1	0	2	1	0	0	5	5	40
			Juv.	0	0	0	0	2	2	1	8	0	1			
Ruffed grouse	Biological Station	1965, 1966	Ad.	9	13	5	0	0	2	0	0	0	0	0	0	30
			Juv.	0	0	0	0	0	0	1	0	0	0			
Franklin's grouse	Biological Station	1966	Ad.	0	7	14	1	1	3	1	1	4	2	0	0	34
			Juv.	0	0	0	0	0	0	0	0	0	0			

Two smears were made. In the spring, heart blood was taken immediately after the bird was shot. During summer and fall, when groups rather than individual birds were encountered, some heart punctures were delayed in an effort to collect as many birds as possible from a group. Some reasonably successful heart blood smears were made as long as an hour after death, but 15 to 20 min was a more realistic time limit to assure good smears with a minimum of post-mortem changes. In a few cases even this brief time was too long.

Blood smears were air-dried. On warm days they dried rapidly without assistance. On cool days and in the early morning it was necessary to wave them in the air.

Bone marrow smears were made by cutting off both ends of the tibio-tarsus, inserting an 18-gauge syringe needle into the marrow cavity, and expressing some marrow onto a slide. Another slide was placed in contact with the first and the two slides were drawn apart. One smear was then air-dried and the second wet-fixed in Schaudinn's fixative.

Tissue smears were made by blotting the cut surface of the tissue on filter paper and then touching it to a slide. They were then air-dried in the same way as blood smears.

Pieces of liver, kidney, lung, and brain were removed, cut so that they were 4 mm or less in thickness, and fixed in Zenker-formol which was prepared in the field from stock Zenker's solution and formalin. Spleens which sometimes exceeded 4 mm in thickness were removed and fixed in their entirety. Because of failure to locate the organ or damage to it by shot, not all of these organs were available for study from every bird collected.

It was found that on days when the temperature was below about

50°F fixation could be delayed for as much as an hour without a great deal of post-mortem change taking place. Tissues were fixed for 6 to 18 hours. On very warm days the shorter period was usually sufficiently long for fixative penetration, but under most conditions about 12 hours resulted in better fixation. After fixation tissues were stored in formaldehyde-calcium, or, if embedding was to be done immediately upon returning to the laboratory, in tap water carried for this purpose. Distilled water was tried, but because of its acidity often resulted in the formation of formaldehyde pigment in tissues.

The above procedure, although yielding in most cases a very good histologic and cytologic picture, was troublesome in two respects. It was inconvenient to have to carry two solutions into the field and mix them before use. Also, if tissues were not removed from the fixative on time, overfixation resulted in excessive hardening and in some cases resulted in loss of affinity for nuclear stains.

Wallington (1955) recommends fixation in 10% formalin, where the tissue can be left indefinitely and, when convenient, secondary fixation in another fixative (such as Zenker-formol) to overcome the deficiencies of formalin fixation. This technique would be ideal for material which had to be fixed in the field. Unfortunately, it was discovered too late for trial in this study.

The date, time, location, sex and age (when these could be readily determined), weight, and the number of birds seen were recorded each time a bird was collected.

Laboratory techniques

Air-dried marrow, blood, and tissue smears were fixed in absolute methyl alcohol and stained in Giemsa 1:50 (Harleco Azure B type),

buffered at pH 6.8, for 1 hour at room temperature. They were then rinsed in buffer solution and blotted dry. Only those which showed heavy infections or those which were to be photographed were later covered with a coverslip mounted on D.P.X. medium. Wet-fixed bone marrow smears were washed in water, transferred to 80% ethyl alcohol containing 0.5% iodine, and then to 70% alcohol for removal of the iodine. They were then placed in water and stained as above or with May-Grünwald-Giemsa (Emmel and Cowdry, 1964), blotted dry, and covered.

Tissues were washed overnight in running tap water, dehydrated in cellosolve (including 0.5% iodine in 80% cellosolve to remove mercury), cleared in benzene, and embedded in paraffin. Two slides of each tissue were routinely stained and examined. More were prepared only to elucidate parasite morphology or for histochemical tests. They were sectioned at 6-8μ and routinely stained with Delafield's hematoxylin-eosin-Azure II (Maximow's stain) as outlined in Emmel and Cowdry (1964). Late in this study the Azure-eosin technique described by Lillie (1954) was tried and found to be generally superior. Other stains employed to study parasites in tissue sections were the Feulgen reaction, for nucleic acids, and iron hematoxylin. The latter was almost always unsuccessful with this material, presumably due to problems of fixation described previously. Perl's Prussian blue reaction and Nile blue A (Pearse, 1960) were used to identify pigments seen in the sections.

Methods of examination

Slides were examined with a Reichert binocular microscope fitted with apochromatic objective lenses of 10, 30, 60, and 100-power and 12.5-power-compensating oculars. A 12 v tungsten filament lamp with transformer provided illumination, except for photographic purposes when a zirconium

arc source was substituted.

Entire smears were scanned using the 10-power objective for detection of microfilaria and trypanosomes. With practice, mature gametocytes of Leucocytozoon also could be readily detected at this magnification. This procedure usually took less than 5 min. Approximately a 5-min search was then made using the 30-power objective for Haemoproteus, Plasmodium, and younger stages of Leucocytozoon, concentrating on the ends and edges of the smear. Finally, a 10-min search was made under oil for very young gametocytes which might have been overlooked under lower magnification.

Gametocyte intensities in smears were obtained by counting gametocytes and mature erythrocytes under oil using the "~~ramparts~~ battlements" method as described in Dormady and Davenport (1963) and keeping count on a hand tally. In the case of Haemoproteus infections 1,000 mature erythrocytes were counted; with Leucocytozoon infections, which were of much lower intensity, 2,000 mature erythrocytes were counted, but the results were expressed as gametocytes per 1,000 erythrocytes, hereafter expressed as gametocytes o/oo. All counts were repeated 5 to 8 days after the initial count, and the two figures were averaged. Replications were close to original counts in the majority of cases.

Tissues were examined for large schizonts with the 10-power objective. The 60-power objective was necessary to verify the presence of hepatic schizonts and gametocytes.

The bone marrow and tissue smears were found to be much less satisfactory and more difficult to interpret than sections. They were not used in this study.

The Atlas of Avian Hematology by Lucas and Jamroz (1961) was very helpful in identifying various blood cell types.

RESULTS

Because of the multitude of protozoan parasites which have been described, many of the descriptive terms used in the literature have become ambiguous. This is especially true of the sporozoans. To avoid confusion a few of the more troublesome terms will be defined as they will be used in the following pages.

The term gametocyte refers to all parasites of the genus Leucocytozoon or Haemoproteus seen intracellularly in blood smears. The term young gametocyte will be used in preference to trophozoite for the smallest intracellular stages seen. Merozoite will be reserved for the final development of schizonts, that is, those parasites released into the blood and seen extracellularly after the rupture of schizonts. Mature, when used in reference to parasites, will refer only to the attainment of full or nearly full size by the parasite and will not imply the attainment of sexual maturity. Adult, when referring to the host, will imply a bird which is at least in its second year.

Description of the parasites

Trypanosoma sp.

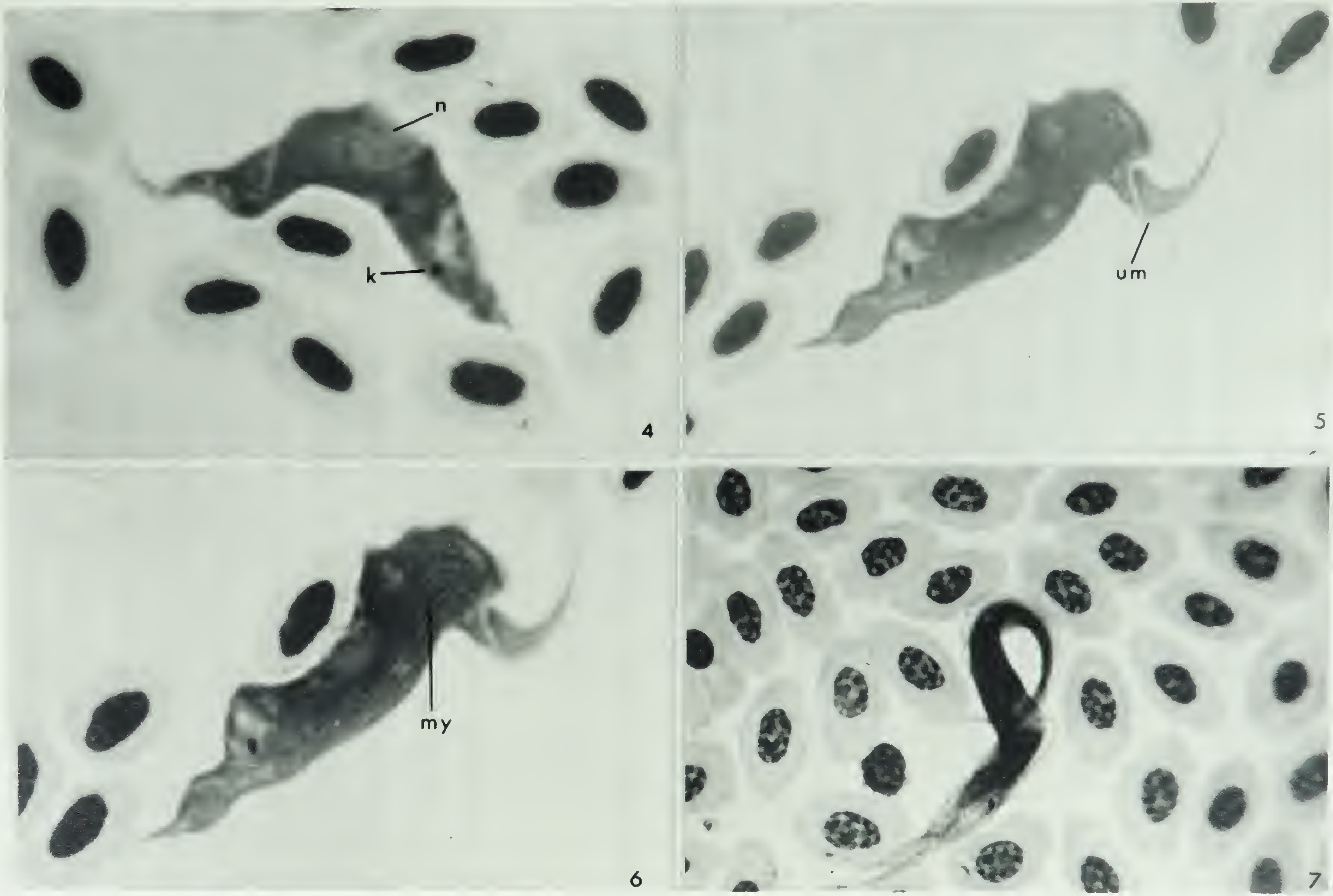
Two morphological types of trypanosomes were seen in the blood smears of both ruffed and Franklin's grouse. A broad form (Figures 4, 5, and 6) which measured from 42 to 48μ long by 8 to 9μ wide was present. The free flagellum was sometimes as long as 9μ but usually appeared shorter. The overall color of the stained parasite was dark blue. The nucleus appeared as a semicircular or triangular area along the same side as the undulating membrane, approximately at the middle of the body. It stained pale pink or remained unstained. The elliptical kinetoplast which was stained brilliant red lay adjacent to the side opposite the undulating membrane, about halfway between the nucleus and the

Figure 4. A typical broad-form trypanosome as seen in a Giemsa-stained blood smear, showing nucleus (n) and kinetoplast (k). 2500X.

Figure 5. A broad-form trypanosome focused to show undulating membrane (um). 2000X.

Figure 6. A broad-form trypanosome showing myonemes (my). 2500X.

Figure 7. A narrow-form trypanosome. Note that no myonemes are visible. 2000X.



n
k
4
um
5
my
6
7

posterior end of the body. Myonemes (Figure 6) were visible in all but the most darkly stained specimens (in which they were obscured), and occurred in 8 to 12 rows. Pale or unstained areas occurred regularly near the kinetoplast and along one side near the undulating membrane. Other pale-staining areas occurred throughout the cytoplasm.

The narrow form (Figure 7) was similar to the above but ranged from 46 to 56μ long by only 3 to 5μ wide, and lacked myonemes. These may have represented more immature forms, but since the sample size was small it is impossible to say whether intermediate forms also existed.

The trypanosomes found in this study can probably be assigned to the species _T. avium_ (Novy and MacNeal, 1905). The two forms seen are within the size range described for _T. avium_, although the free flagellum of the specimens seen in this study seems to be shorter, possibly an artefact through damage in smear preparation. Novy and MacNeal described forms with 6 to 8 myonemes. Dorney and Todd (1960) also found two morphological forms in ruffed grouse, one with myonemes and one without, both between 48 and 60μ long.

Haemoproteus sp.

In blood smears of ruffed and Franklin's grouse, gametocytes of _Haemoproteus_ were found in all stages of development in red blood cells. It was common to see very young and mature gametocytes in the same smear. On at least one occasion young gametocytes were seen in a polychromatic erythroblast (Figure 16), indicating that developmental stages of the erythrocyte series are also invaded. Mature gametocytes often completely encircled the host-cell nucleus in both ruffed and Franklin's grouse. Slight enlargement of the host cell was sometimes evident. Nuclei which were surrounded by mature parasites often appeared reduced in size. The parasites were usually close to, but not quite touching, the nucleus and were always quite closely applied to

the erythrocyte membrane. On occasion host-cell nuclei were displaced to one side (Figure 18), and in a few instances were pushed by the parasite directly against the cell membrane. This tendency seemed to be more common in cells containing microgametocytes (Figure 18).

Macrogametocytes (Figures 14 and 15) stained dark blue and had a definite pink to red-staining nucleus and alveolar cytoplasm. Microgametocytes (Figure 13) were paler staining with an indefinite nuclear area. Their cytoplasm was also alveolar, but this was not so noticeable as in macrogametocytes. Pigment granules were present in both sexes. Figure 44 graphically illustrates the number of pigment granules in gametocytes from ruffed, spruce, and Franklin's grouse. Granules were counted only in gametocytes which occupied at least three-fourths of the cytoplasm of the host cell. The ratios of macrogametocytes to microgametocytes in the blood varied from 1.3:1 to 21:1.

Very young gametocytes (Figures 8 and 9) appeared as fusiform or elliptical objects in the cytoplasm of erythrocytes. There was a prominent, round, deep-red-staining nucleus near one end. The cytoplasm usually stained more deeply at the end opposite the nucleus. The parasites were usually near one pole of the erythrocyte at this stage. Growth appeared to proceed toward the opposite pole until the parasite occupied most of one side of the host cell. At this stage it was sausage-shaped and had pigment granules concentrated at the growing end (Figures 12 and 13). This end was usually narrower and less regular in outline. The nucleus was no longer as prominent or dark-staining and occupied a larger area toward the center of the parasite. Sometimes smaller, more intensely-staining red bodies were seen peripheral to the nucleus at this stage. After having reached the limits of the long axis of an erythrocyte, both ends of the growing gametocyte appeared to grow toward one another. Infections with two gametocytes in an erythrocyte were seen (Figure 17).

Figure 8. Very young gametocytes of *Haemoproteus* in grouse erythrocytes. These probably represent development in the first few hours after entering the host cell. 3000X.

Figure 9. Young gametocyte of *Haemoproteus*, slightly later stage than in Figure 8. Note the ring-like morphology. 3500X.

Figure 10. Young gametocyte of *Haemoproteus*, similar to Figure 9, but with the bulk of the densely-stained protoplasm of the parasite concentrated at one end. 3500X.

Figure 11. Two young gametocytes of *Haemoproteus*. The one on the right represents the smallest form seen in this study. 3500X.

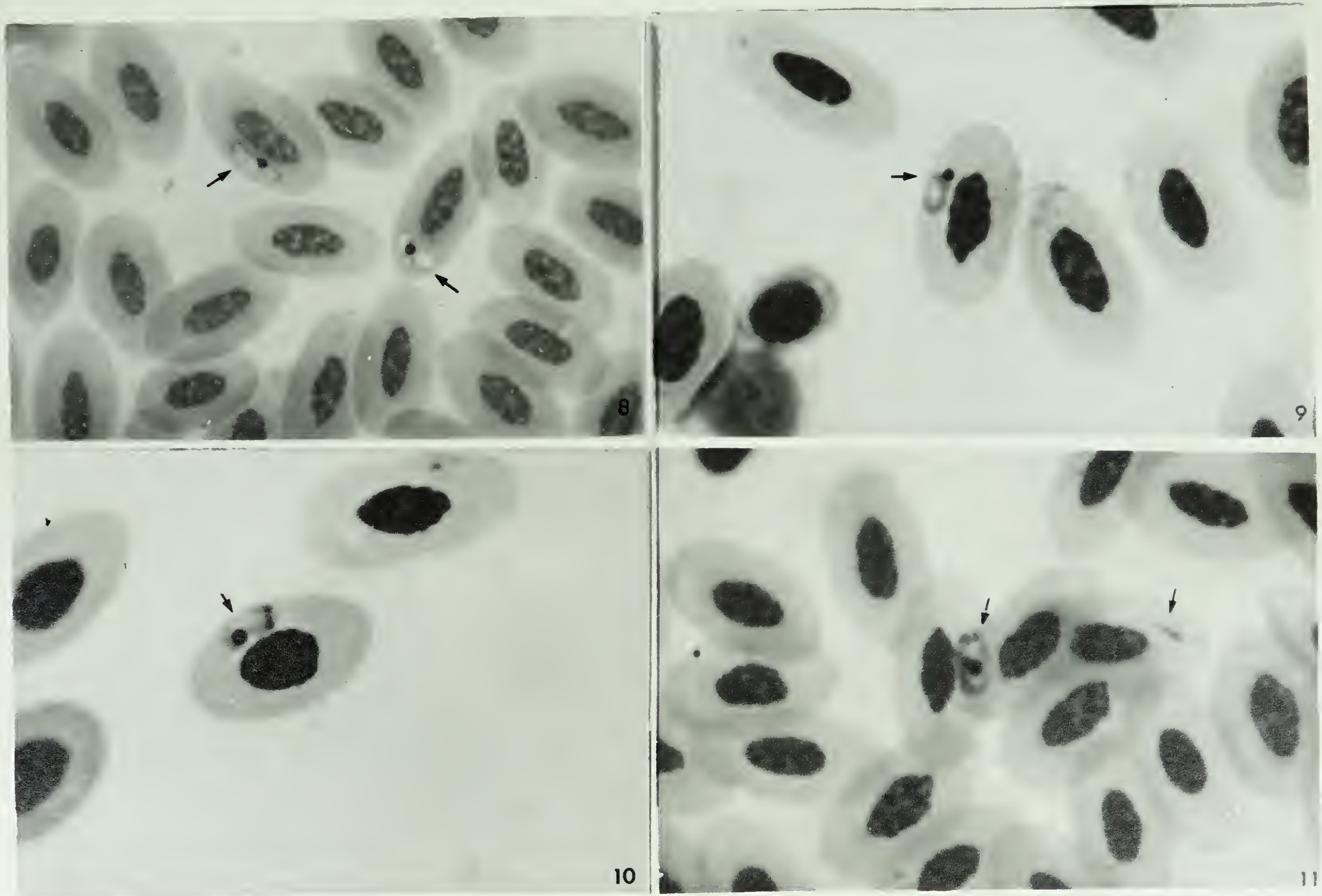

8
9
10
11

Figure 12. An intermediate stage in the development of a gametocyte of _Haemoproteus_. 3000X.

Figure 13. A microgametocyte of _Haemoproteus_. A pale-staining endosome (end) can be seen within the indistinct nucleus. 3000X.

Figure 14. A macrogametocyte of _Haemoproteus_. Before the parasite completely surrounds the nucleus, the dark pigment granules appear to be concentrated near the ends of the parasite. 3000X.

Figure 15. A macrogametocyte of _Haemoproteus_. 3000X.

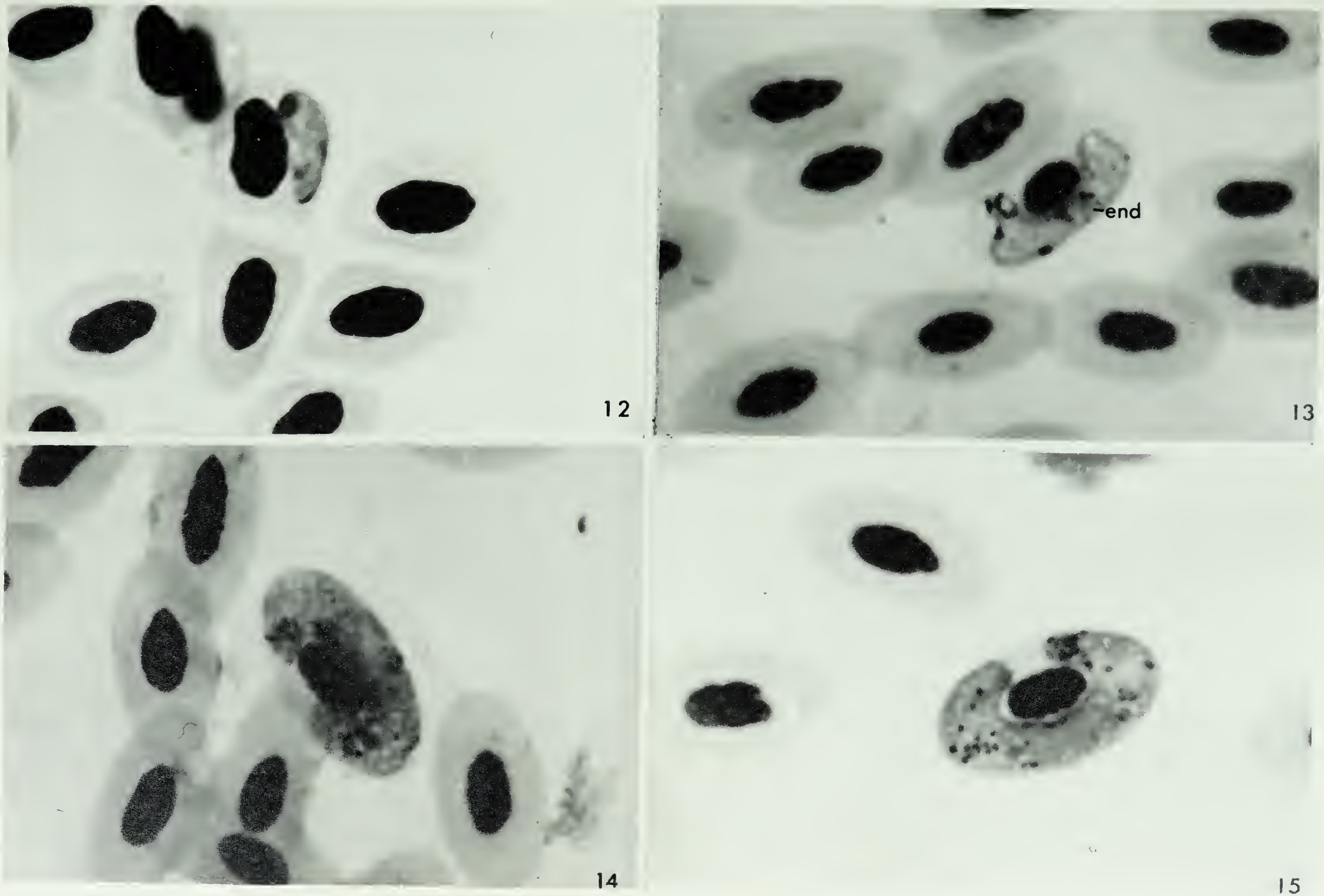
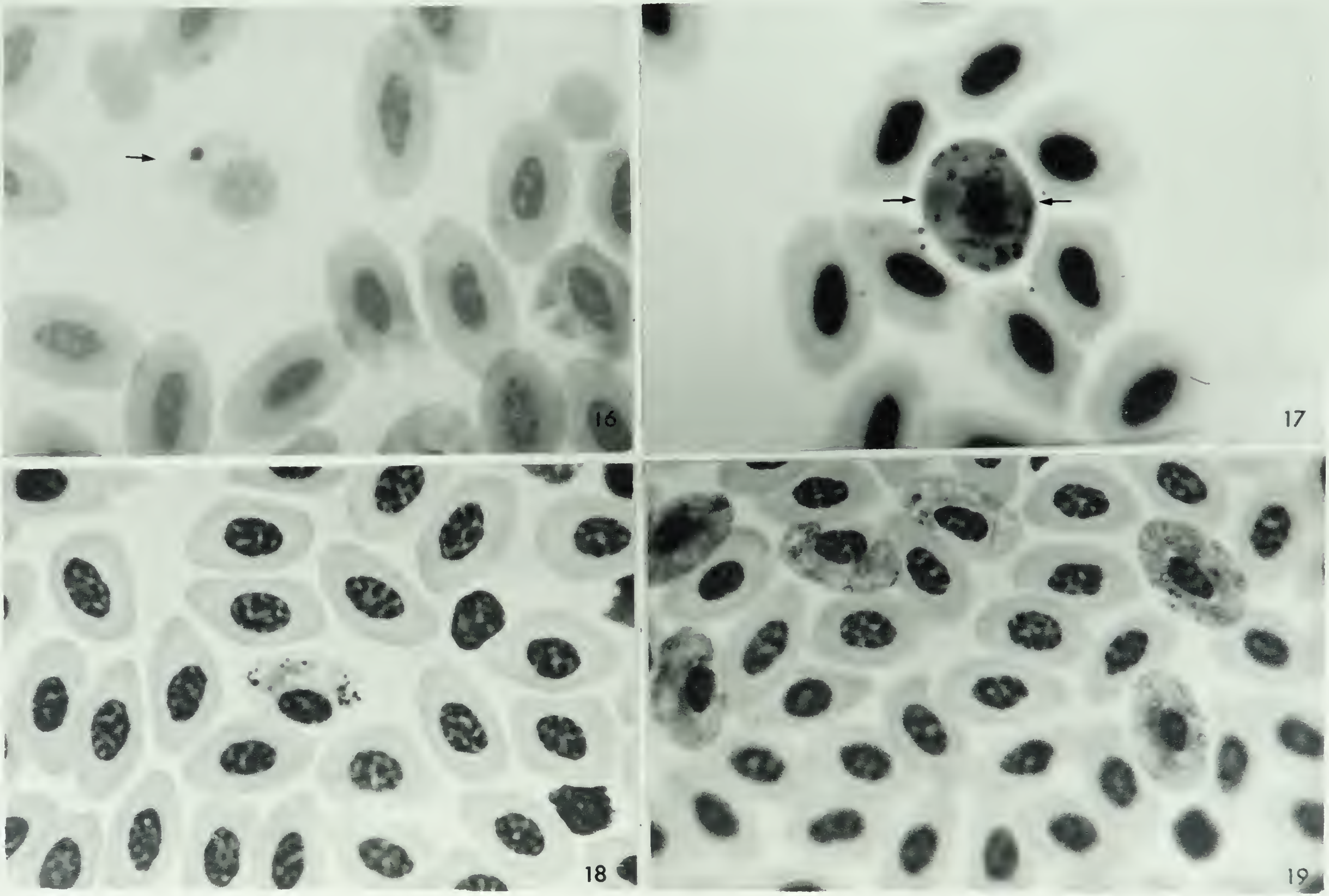

12
13
-end
14
15

Figure 16. A young gametocyte of Haemoproteus in a polychromatic erythroblast of ruffed grouse. 2000X.

Figure 17. Two macrogametocytes of Haemoproteus within a single erythrocyte. The two parasite nuclei (arrows) are quite distinct. 2000X.

Figure 18. An immature microgametocyte of Haemoproteus. Note the displacement to one side of the host-cell nucleus. 2000X.

Figure 19. Photomicrograph representing the maximum density of gametocytes of Haemoproteus seen in a ruffed grouse blood smear. 2000X.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17
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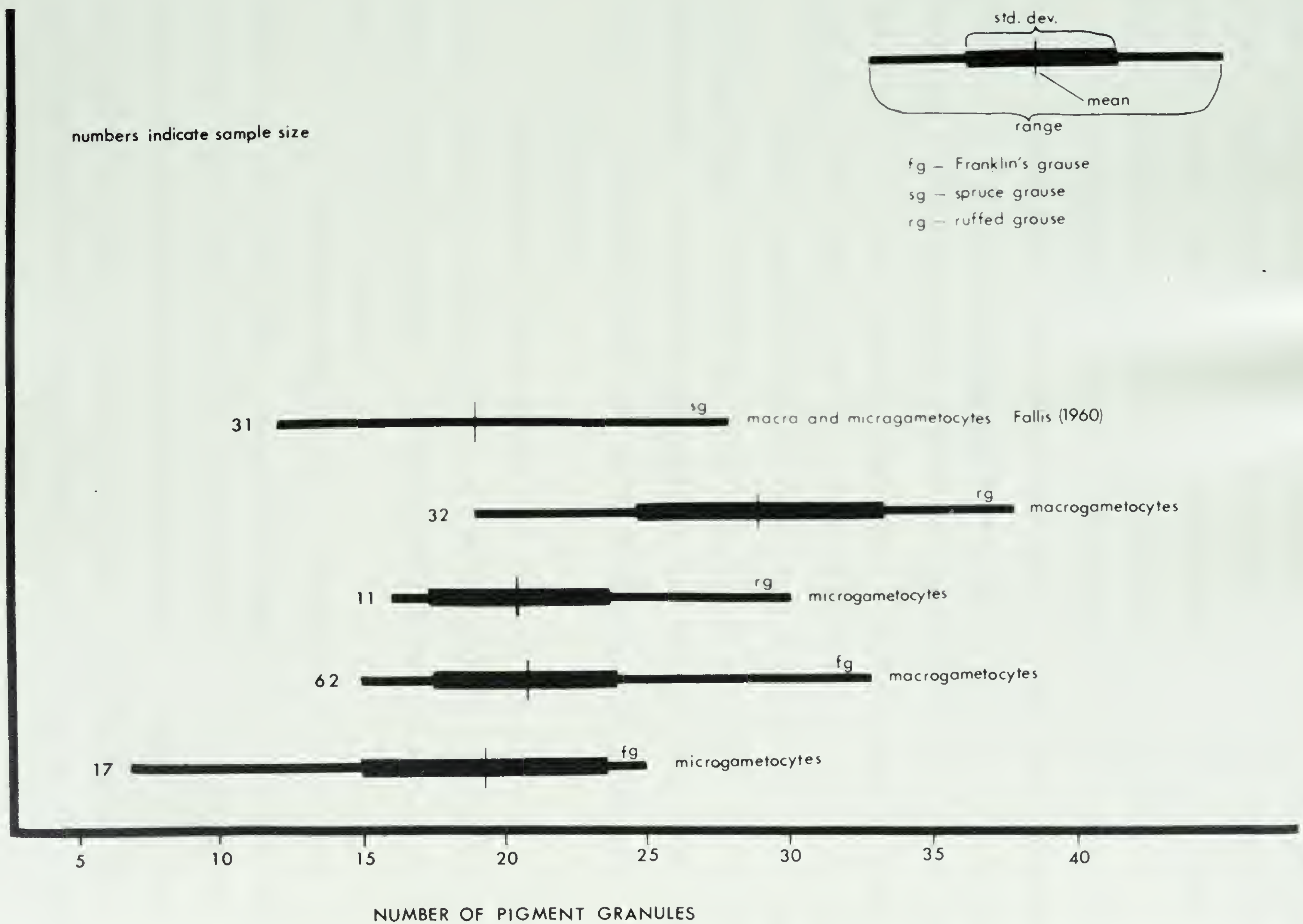
19

Figure 44. Numbers of pigment granules in gametocytes of Haemoproteus.



std. dev.
mean
range
fg – Franklin's grause
sg – spruce grause
rg – ruffed grouse
numbers indicate sample size
31
sg
macra and micragametocytes
Fallis (1960)
32
rg
macrogametocytes
11
rg
microgametocytes
62
fg
macrogametocytes
17
fg
microgametocytes
5
10
15
20
25
30
35
40
NUMBER OF PIGMENT GRANULES

Fallis and Bennett (1960) described _H. canachites_ n. sp. from a spruce grouse in Ontario. This genus has been recorded from various grouse (Clarke, 1935; Oliger, 1940; Fallis, 1945; Fowle, 1946; Borg, 1953; Bendell, 1955; Holmes and Boag, 1965), but only Sambon (1908) and Fantham (1910a) assigned a species (_H. mansoni_) to specimens from the red grouse.

The _Haemoproteus_ seen in ruffed and Franklin's grouse in the present study is morphologically indistinguishable from that described by Fallis and Bennett (1960) or from the _Haemoproteus_ described by Clarke (1935), although the latter claimed that in his material the gametocytes did not cause enlargement of the host cells. A ruffed grouse was successfully infected with _H. canachites_ from a spruce grouse in experiments performed by Fallis and Bennett.

Bennett, Garnham and Fallis (1965) have suggested that a new genus, _Parahaemoproteus_, be erected for those organisms which undergo sporogony in ceratopogonids, as opposed to _Haemoproteus_ which undergoes sporogony in hippoboscids. _H. canachites_ would then become _Parahaemoproteus canachites_.

Leucocytozoon sp.

Gametocytes of this organism were found in all stages of development in blood smears from ruffed grouse, although the earliest developmental forms were encountered only rarely. No developmental forms were seen in blood smears from Franklin's grouse.

Both rounded and elongate forms occurred (Figures 24 and 25), the former being much more common. Rounded and elongate forms sometimes occurred in the same blood smears, but this was not usually the case. Twenty-five rounded macrogametocytes were from 13 to 19μ long ($\bar{x}$ = 16.8; s = 1.7) and had a length:width ratio of from 1.1:1 to 1.4:1. Fifteen elongate macrogametocytes were 16 to 28μ long ($\bar{x}$ = 22.2; s = 2.3) and had a length:width

ratio of from 1.5:1 to 5:1 (only two of those measured had less than a 2:1 ratio). All measurements were taken from parasites of central Alberta ruffed grouse. Microgametocytes were too scarce to permit a detailed analysis. Those which were encountered did not appear to be very different in size from macrogametocytes. Figure 45 shows the size of Leucocytozoon gametocytes found in this study compared with those reported in the literature from other grouse.

The alveolar cytoplasm of mature microgametocytes stained medium to deep blue. The nucleus stained pink and occupied a small area near the center of the parasite. Microgametocytes were pale-staining with a diffuse, pink-staining, centrally-located nucleus. The parasite's nucleus usually extended from the host-cell nucleus to the opposite membrane of the host cell. The cytoplasm of macrogametocytes sometimes appeared to contain pigment granules. Host-cell nuclei were concave on the side adjacent to the parasite and appressed the cell membrane.

Cells containing mature gametocytes often had pale-staining, actiniform, cytoplasmic extensions, although these were usually lost in preparing the smear. They were very prominent in parasitized cells of fresh blood examined with a phase-contrast microscope.

Younger gametocytes were round and blue-staining, with a pink to red-staining, centrally-located nucleus. The cytoplasm was stained most darkly at the periphery. None of the invaded cells resembled erythrocytes even though intracellular parasites no larger than about 2μ were seen in blood smears (Figure 20). The cells in which these early stages were seen appeared to be erythroblasts or lymphocytes, or both types.

Growth was seen as enlargement of the parasite with concurrent flattening of the host-cell nucleus (Figures 22 and 23). Cells invaded by

Figure 45. Lengths of macrogametocytes of Leucocytozoon found in grouse blood smears.

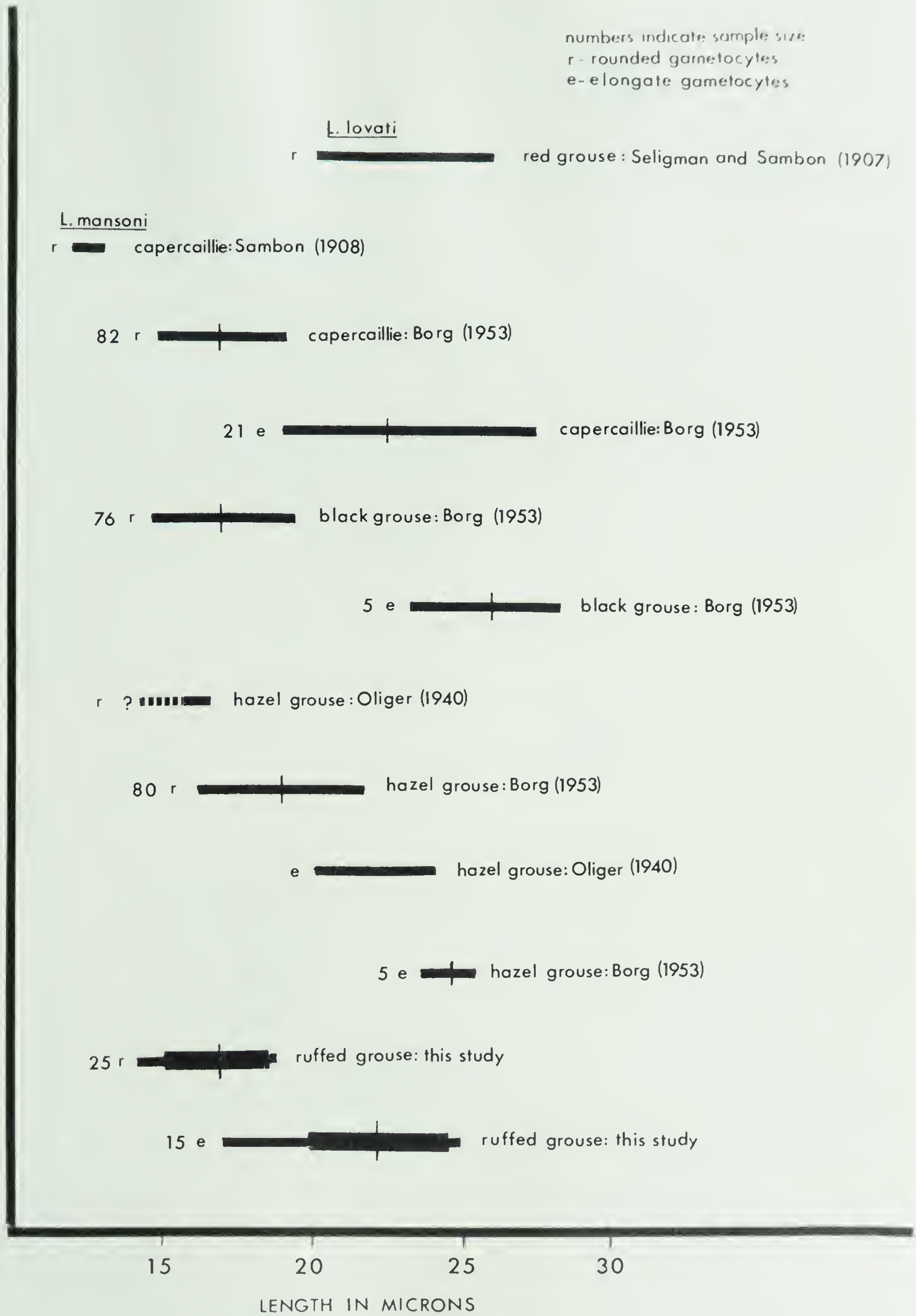

numbers indicate sample size
r - rounded gametocytes
e - elongate gametocytes
L. lovati
r red grouse : Seligman and Sambon (1907)
L. mansoni
r capercaillie: Sambon (1908)
82 r capercaillie: Borg (1953)
21 e capercaillie: Borg (1953)
76 r black grouse: Borg (1953)
5 e black grouse: Borg (1953)
r ? hazel grouse: Oliger (1940)
80 r hazel grouse: Borg (1953)
e hazel grouse: Oliger (1940)
5 e hazel grouse: Borg (1953)
25 r ruffed grouse: this study
15 e ruffed grouse: this study
15
20
25
30
LENGTH IN MICRONS

Figure 20. Two merozoites (m) or very young gametocytes of Leucocytozoon, one intracellular and one extracellular. The morphological appearance of the host-cell nucleus (hn) is that of an erythroblast or lymphocyte. These are the earliest stages seen in any blood smear. 3000X.

Figure 21. Two young gametocytes (g) of Leucocytozoon within a single host cell. Note vacuole (v) surrounding the parasites and appearance of the host-cell nucleus, characteristic of cells parasitized by Leucocytozoon. 3000X.

Figure 22. Development of gametocyte of Leucocytozoon. Note distorted host-cell nucleus, the parasite nucleus (pn), and endosome (end). 3000X.

Figure 23. Development of gametocyte of Leucocytozoon, similar to Figure 22, but somewhat more developed. 3000X.

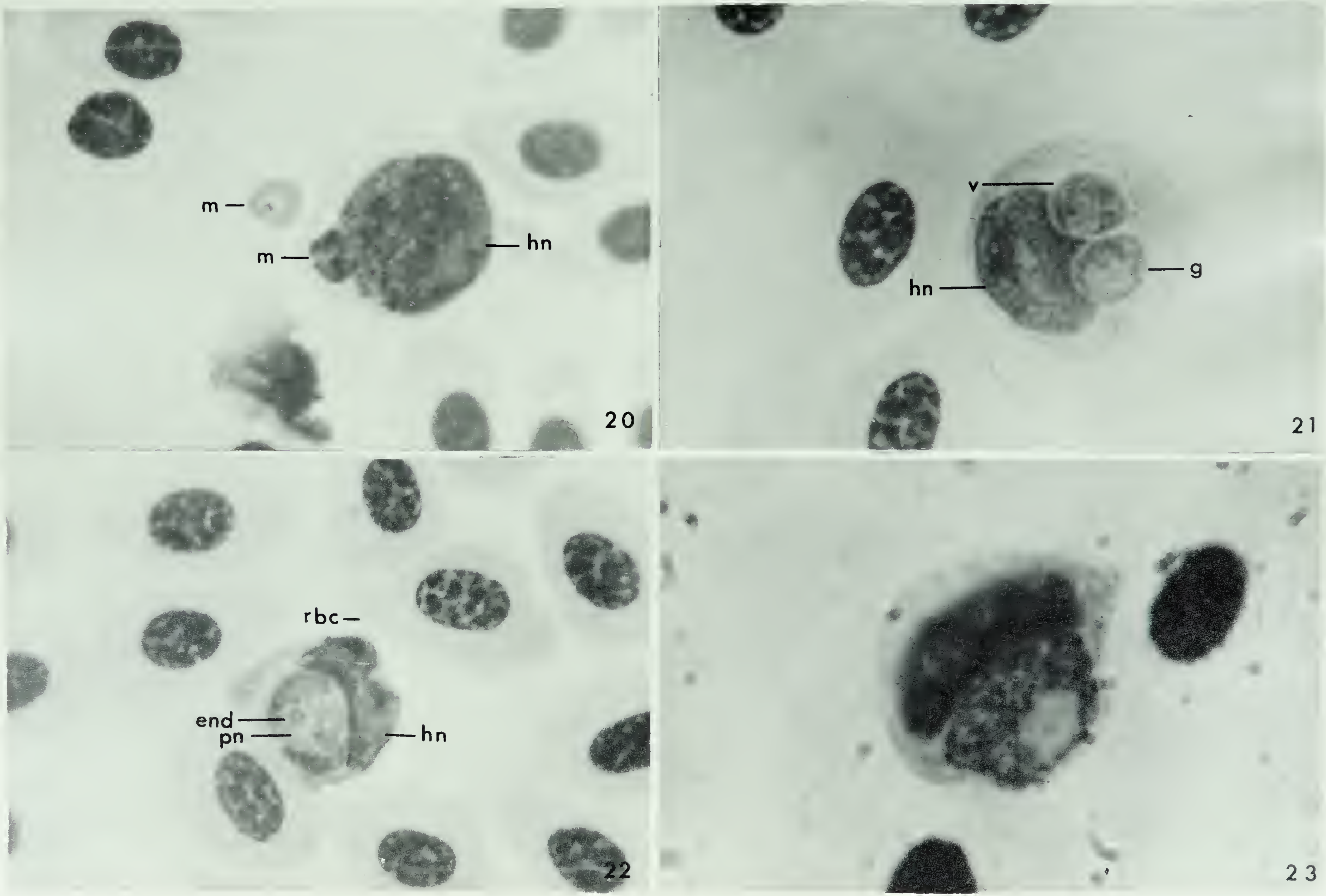

m
m
hn
20
v
hn
g
21
rbc
end
pn
hn
22
23

Figure 24. A mature, rounded macrogametocyte of _Leucocytozoon_. Note the host-cell nucleus (hn) and pointed extensions of the host-cell cytoplasm (cyt). 3000X. Insert shows the elongate form of macrogametocyte of _Leucocytozoon_. 2000X.

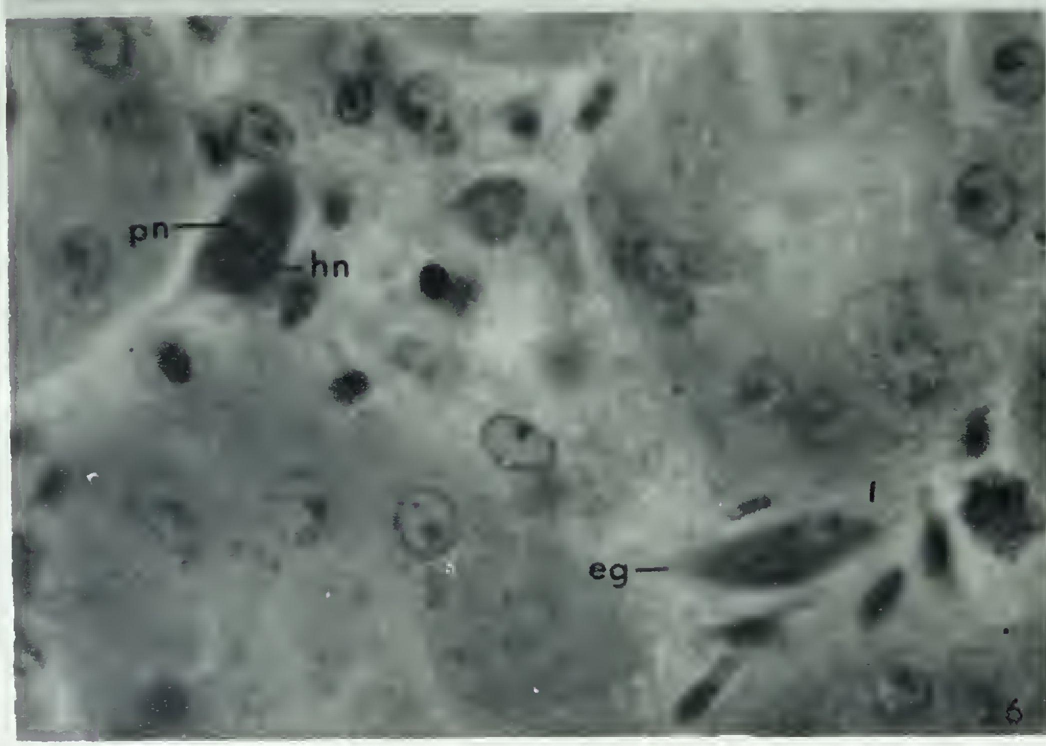
Figure 25. A mature, rounded microgametocyte. The somewhat ill-defined parasite nucleus can be seen. 3000X.

Figure 26. Elongate gametocytes of _Leucocytozoon_ as they appear in a kidney section. 2000X.

Figure 27. Rounded gametocytes as they appear in an hepatic sinusoid. 2000X.


hn
cyt
24


hn
pn
25


pn
hn
eg
6


27

two young gametocytes were observed (Figure 21), and on one occasion three parasites were seen in a single cell. There never appeared to be more than one mature gametocyte per cell however, and the fate of multiple infections remains unknown.

The species of Leucocytozoon found in this study resembles L. bonasae described from Ontario ruffed grouse by Clarke (1935), L. mansoni described from capercaillie by Sambon (1908), and L. lovati described from red grouse by Seligmann and Sambon (1907). Clarke noted the similarities between L. bonasae and the other two species, but said that L. bonasae differed "in its oval shape and in the reduced length of the fusiform ends of the host cell." These criteria, however, are poor as the shape of the parasite and length of the fusiform ends of the host cell are features which are easily altered in the preparation of blood smears; indeed, enough variations were seen in ruffed grouse smears from the present study to overlap all of the above species. The fusiform ends of the host cell in particular seem to be fragile and subject to destruction during smear preparation. They seldom appeared to be long in fixed smears, but on two occasions they were observed in fresh blood as extremely long. Until cross-transmission studies are done the exact determination of the species of Leucocytozoon in ruffed and Franklin's grouse cannot be stated with certainty. On the basis of morphology alone it would seem that there is no reason to think the parasite differs from L. lovati.

Prevalence of protozoa in blood smears

Usable peripheral blood smears were obtained from 30 ruffed grouse from central Alberta. Thirty-two birds yielded usable heart blood smears. Since no important differences were noted in protozoan parasitemia between peripheral and heart blood, the results from examinations of both were combined

giving a total sample of blood smears from 35 different ruffed grouse from central Alberta. Table II summarizes the extensity of infections found in these smears.

The intensity of infection was determined only for the heaviest infections encountered. Only one moderately heavy infection of Leucocytozoon was found in these blood smears. This was in an adult male ruffed grouse collected in the Swan Hills of Alberta on May 28, 1966. The infection rate was 2 gametocytes per 1,000 erythrocytes. Two moderately heavy infections with Haemoproteus were encountered. The first was from an 11-week-old bird collected on August 20, which had 13 gametocytes per 1,000 erythrocytes. The second bird, an adult female collected August 23, had 3 gametocytes per 1,000 erythrocytes.

Trypanosomes were found in greatest abundance in the same adult male that exhibited the heaviest parasitemia of Leucocytozoon. There were about nine trypanosomes in one smear from this bird. In most trypanosome-positive birds only one or two trypanosomes were found per blood smear.

The extensity of infections in ruffed grouse from the R. B. Miller Biological Station is shown in Table III. None of these birds showed a high parasitemia of any hematozoon.

The extensity of infections found in Franklin's grouse from the R. B. Miller Biological Station is shown in Table IV. Three birds had moderately high parasitemias of Leucocytozoon, but only two of these smears were thin enough to permit counting. One smear collected June 1, 1966, had a count of 1 gametocyte per 1,000 erythrocytes. No gametocytes were seen in counting 2,000 erythrocytes in a smear from the other bird collected June 7, 1966. Haemoproteus infections in Franklin's grouse were more extensive than in ruffed grouse. Five of the most heavily infected birds had 16, 25, 32, 51,

Table II. Incidence of protozoan infections in blood smears of 35 ruffed grouse from central Alberta.

	May		June		July		August		September		October			
	1-15	16-31	1-15	16-30	1-15	16-31	1-15	16-31	1-15	16-30	1-15	16-31	Total	%
							Leucocytozoon sp.							
Ad.	6/6	1/1	2/2	-	1/1	-	2/2	1/1	-	-	1/3	2/5	13/13	100
Juv.	-	-	-	-	0/2	1/2	0/1	5/8	-	0/1			6/14	43
Total	6/6	1/1	2/2	-	1/3	1/2	2/3	6/9	-	0/1	1/3	2/5	22/35	60
							Haemoproteus sp.							
Ad.	0/6	1/1	0/2	-	0/1	-	1/2	1/1	-	-	0/3	0/5	3/13	23
Juv.	-	-	-	-	0/2	0/2	1/1	2/8	-	0/1			3/14	21
Total	0/6	1/1	0/2	-	0/3	0/2	2/3	3/9	-	0/1	0/3	0/5	6/35	17
							Trypanosoma sp.							
Ad.	0/6	1/1	2/2	-	0/1	-	0/2	0/1	-	-	1/3	0/5	3/13	23
Juv.	-	-	-	-	0/2	0/2	0/1	1/8	-	0/1			1/14	7
Total	0/6	1/1	2/2	-	0/3	0/2	0/3	1/9	-	0/1	1/3	0/5	5/35	14

Concurrent

Leucocytozoon + *Haemoproteus* 4   *Leucocytozoon* + *Trypanosoma* 5   *Haemoproteus* + *Trypanosoma* 3   All three genera 3

Table III. Incidence of protozoan infections in blood smears of 30 ruffed grouse from the vicinity of the R. B. Miller Biological Station.

	May		June		July		August		September		October			
	1-15	16-31	1-15	16-30	1-15	16-31	1-15	16-31	1-15	16-30	1-15	16-31	Total	%
						Leucocytozoon sp.								
Ad.	5/9	5/14	4/5	-	-	1/2	-	-	-	-	-	-	15/30	50
Juv.	-	-	-	-	-	-	-	1/1	-	-	-	-	1/1	100
Total	5/9	5/14	4/5	-	-	1/2	-	1/1	-	-	-	-	16/31	52
						Haemoproteus sp.								
Ad.	2/9	4/14	2/5	-	-	0/2	-	-	-	-	-	-	8/30	27
Juv.	-	-	-	-	-	-	-	0/1	-	-	-	-	0/1	0
Total	2/9	4/14	2/5	-	-	0/2	-	0/1	-	-	-	-	8/31	26
						Trypanosoma sp.								
Ad.	2/9	5/14	3/5	-	-	1/2	-	-	-	-	-	-	11/30	37
Juv.	-	-	-	-	-	-	-	0/1	-	-	-	-	0/1	0
Total	2/9	5/14	3/5	-	-	1/2	-	0/1	-	-	-	-	11/31	35

Concurrent

Leucocytozoon + *Haemoproteus* 5 *Leucocytozoon* + *Trypanosoma* 8 *Haemoproteus* + *Trypanosoma* 5 All three genera 4

Table IV. Incidence of protozoan infections in blood smears of adult Franklin's grouse from the vicinity of the R. B. Miller Biological Station

	May		June		July		August		September		October			
	1-15	16-31	1-15	16-30	1-15	16-31	1-15	16-31	1-15	16-30	1-15	16-31	Total	%
						Leucocytozoon sp.								
Ad.	-	6/7	12/14	0/1	1/1	3/3	1/1	0/1	2/4	1/2	-	-	26/34	76
						Haemoproteus sp.								
Ad.	-	7/7	12/14	0/1	0/1	2/3	1/1	1/1	3/4	2/2	-	-	28/34	82
						Trypanosoma sp.								
Ad.	-	2/7	3/14	0/1	0/1	2/3	1/1	0/1	2/4	0/2	-	-	10/34	29

Concurrent

Leucocytozoon + *Haemoproteus* 21 *Leucocytozoon* + *Trypanosoma* 7 *Haemoproteus* + *Trypanosoma* 8 All three genera 7

and 30 gametocytes per 1,000 erythrocytes. These birds were captured on May 21, June 1, June 1, June 1, and June 9, respectively. Parasitemias of similar magnitude were seen in smears from birds captured May 21, June 8, June 13, and July 19. All except one of the above smears were from male birds; however, 67% of the adult birds sampled were males.

Of special interest were two birds bled on more than one occasion. One adult male bled on June 1, had a low parasitemia of Leucocytozoon and a high parasitemia of Haemoproteus. On August 12, it still had a low parasitemia of Leucocytozoon, a very low parasitemia of Haemoproteus, and in addition, some trypanosomes were present. Another adult male bled on June 8, had a very low parasitemia of Leucocytozoon and a high parasitemia of Haemoproteus. On August 28, the bird showed no Leucocytozoon and a markedly decreased number of Haemoproteus in the blood. A trypanosome was seen at this time. On September 13, no Leucocytozoon gametocytes were found, Haemoproteus was very scarce, but trypanosomes were more numerous than on August 28.

Results of tissue examinations

Usable tissue samples consisted of 34 spleen, 30 brain, 37 liver, 30 kidney, and 30 lung dissected from ruffed grouse collected in central Alberta.

Schizonts of Haemoproteus were not definitely identified in any of the sections examined in this study. The occurrence of Leucocytozoon in ruffed grouse tissues is shown in Table V. In addition to gametocytes, which could be located in the blood vessels and sinuses of the tissues examined (Figures 26 and 27), two types of schizonts are listed.

The larger megaloschizonts (Figures 35 to 39) were found only in kidney sections of two birds. The smaller schizonts were found in liver sections of 28 grouse and may have occurred in others. These occurred not

Table V. Incidence of Leucocytozoon in blood smears and tissue sections of ruffed grouse from central Alberta.

Bird No.	Blood Gametocytes	Liver Gametocytes	Schi-zonts	Kidney Gametocytes	Schi-zonts	Spleen Gametocytes	Schi-zonts	Lung Gametocytes	Schi-zonts
1	+	–	S	+	S	0	0	+	–
2	+	–	S	0	0	–	–	+	–
3	+	–	S	+	–	–	–	+	–
4	+	+	–	–	–	0	0	0	0
5	+	–	–	–	–	0	0	–	–
6	0	0	0	0	0	–	–	0	0
7	+	–	–	–	–	–	–	–	–
8	0	–	S	0	0	–	–	+	–
9	0	+	S	+	M	0	0	0	0
10	+	–	S	–	–	–	–	+	–
11	+	–	S	–	M	–	–	+	–
12	+	–	S	+	–	+	–	–	–
13	–	–	S	0	0	–	–	+	–
14	–	+	S	0	0	–	–	–	–
15	+	0	0	+	–	–	–	+	–
16	+	+	S	+	–	–	–	0	0
17	–	0	0	–	–	–	–	+	–
18	+	–	S	–	–	–	–	0	0
19	–	–	S	–	–	–	–	+	–
20	+	–	S	0	0	–	–	0	0
21	+	+	–	–	–	0	0	0	0
22	+	+	–	+	–	–	–	0	0
23	+	–	–	0	0	–	–	+	–
24	–	–	S	0	0	–	S	–	–
25	+	+	S	+	–	+	–	+	–
26	–	–	S	0	0	–	S	–	–
27	–	–	S	–	–	–	–	+	–
28	+	–	S	+	–	+	–	+	–
29	+	–	S	–	–	–	–	+	–
30	–	–	S	–	S	–	–	–	–

Table V continued.

Bird No.	Blood Gametocytes	Liver Gametocytes	Schi-zonts	Kidney Gametocytes	Schi-zonts	Spleen Gametocytes	Schi-zonts	Lung Gametocytes	Schi-zonts
31	+	–	S	+	–	–	–	+	–
32	0	–	S	–	–	–	–	0	0
33	0	–	–	–	–	–	–	+	–
34	–	–	S	–	S	–	–	–	–
35	–	–	S	–	–	–	–	0	0
36	–	–	S	–	–	–	–	+	–
37	–	–	S	–	–	0	0	+	–
38	+	–	S	–	–	–	–	–	–
39	–	–	S	–	–	–	–	+	–
40	+	–	S	0	0	–	–	–	–

0 = not available for study S = small schizonts M = megaloschizonts + = positive – = negative

only in the parenchymal cells of the liver (Figures 28 to 31), but also in the Kupffer cells and macrophages (Figures 32 and 33). Small schizonts were also found in macrophages in spleen and kidney.

Megaloschizonts found in this study were all relatively mature. They appeared to occur in the lumen of kidney tubules, or in the space between tubules, or in blood vessels. They often appeared to be contained within a sheath of reticular fibers. The maturing merozoites were usually divided into distinct groups or cytomeres by one or more cleft-like vacuoles. Often two adjacent cytomeres appeared to be at different stages of development (Figure 38). Some of the megaloschizonts contained a large nucleoid body, which may have been the greatly hypertrophied nucleus of the host cell. In some sections as many as six megaloschizonts could be seen in one field under the 10-power-objective lens; however, the distribution through the kidney was irregular, and many sections showed few or no megaloschizonts.

Small schizonts in the liver and elsewhere were limited in size to about 7 or 8μ; the largest were seen in Kupffer cells. Because of the large numbers of small lymphoid cells present in the livers of infected birds, it was often difficult to distinguish between the nuclei of these cells, which even infiltrated the spaces between adjacent parenchymal cells, and the smaller schizonts. Pressure created by the growing schizonts often caused the nucleus of the host cell to assume a kidney-bean shape. In parenchymal cells a vacuole surrounded the schizonts. In Kupffer cells the host-cell cytoplasm was indistinct and its nucleus often hypertrophied. Its chromatin would be widely dispersed and the cell would have an enlarged, pale appearance (Figure 32).

In addition to the parasites themselves many of the organs examined contained varying amounts of pigment. Thirty-six of the 37 livers examined contained lipofuscin. In unstained sections this pigment occurred as various-

Figure 28. An early hepatic schizont (hs) of <u>Leucocytozoon</u>. Liver parenchymal cell (lpc); nucleus of red blood cell (rbc). 3000X.

Figure 29. An early hepatic schizont of <u>Leucocytozoon</u>, similar to Figure 28. The vacuole (v) surrounding one of the parasites is clearly seen. Dark-staining clumps of lipofuscin (lf) are also present. 3000X.

Figure 30. Two young hepatic schizonts of <u>Leucocytozoon</u>. The one on the left (out of focus) has four clumps of chromatin in a diamond pattern. 3500X.

Figure 31. An hepatic schizont of <u>Leucocytozoon</u> in some process of division. 3000X.

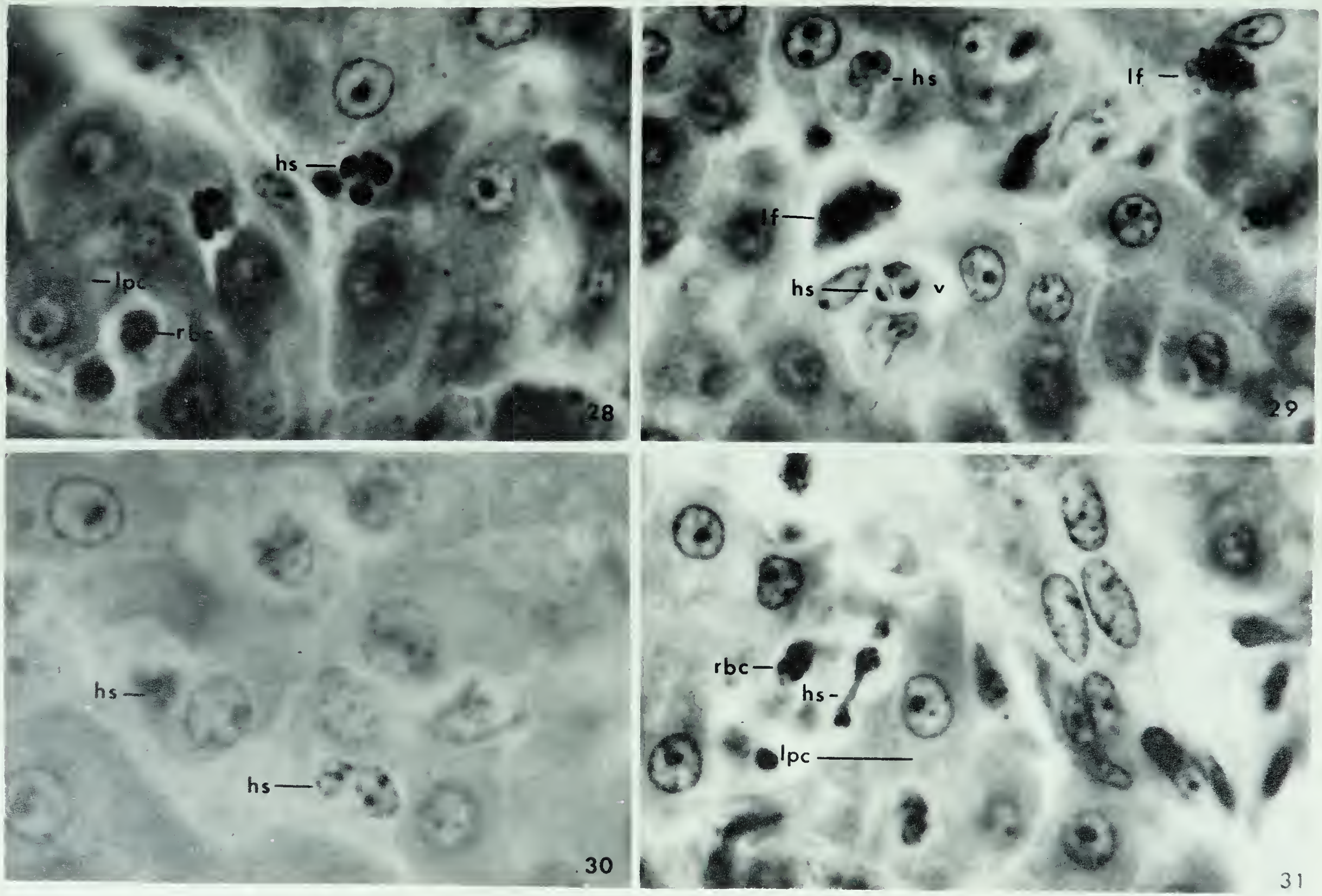
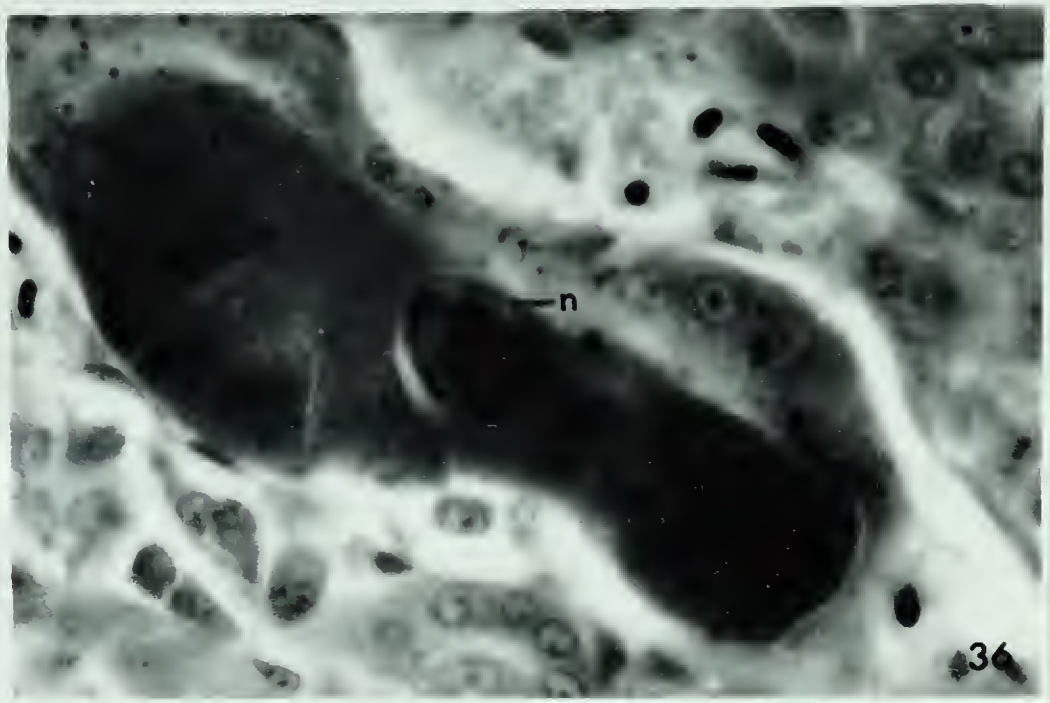

hs
-lpc
rbc
28
- hs
lf
lf
hs
v
29
hs
hs
30
rbc
hs-
lpc
31

Figure 32. A schizont (s) of Leucocytozoon in a liver macrophage or Kupffer cell. To the right of the parasite is a large clump of lipofuscin. 3000X.

Figure 33. A drawing of a large macrophage seen in a grouse kidney. The macrophage nucleus (hn) is at the right. This cell seemed to contain several islands of segmenting parasitic material. Approximately 15000X.

Figure 34. A schizont (s) in an epithelial cell of a kidney tubule. This might be a young stage in the development of a megaloschizont. Note the lack of basophilia of the parasite. Iron-hematoxylin-eosin. 5000X.

Figure 35. A portion of a megaloschizont of Leucocytozoon which appears to be within capillary of the kidney. What appear to be nuclei of endothelial cells (en) can be seen, and the parasite seems to partly surround a deformed kidney tubule (kt). 3000X.



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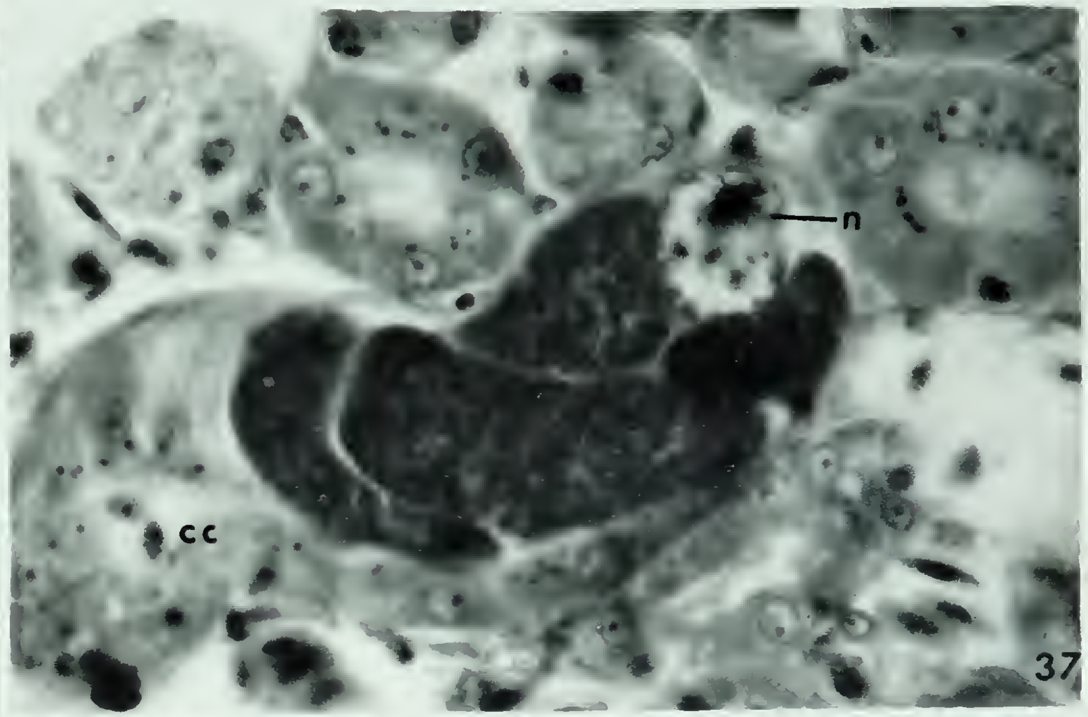

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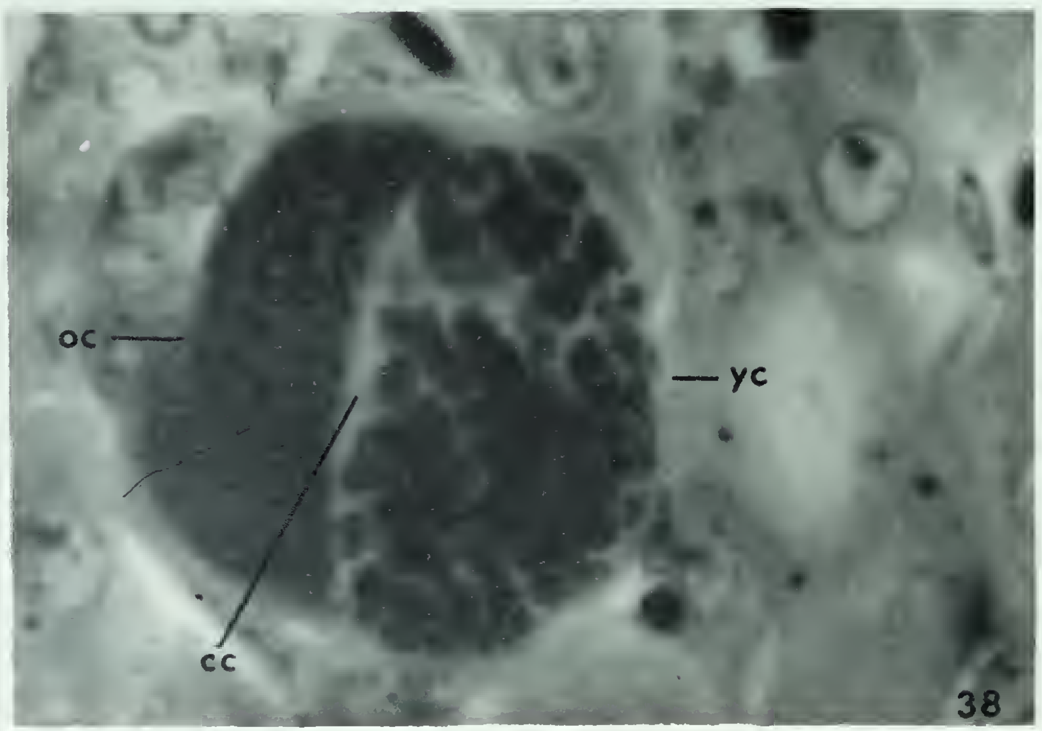

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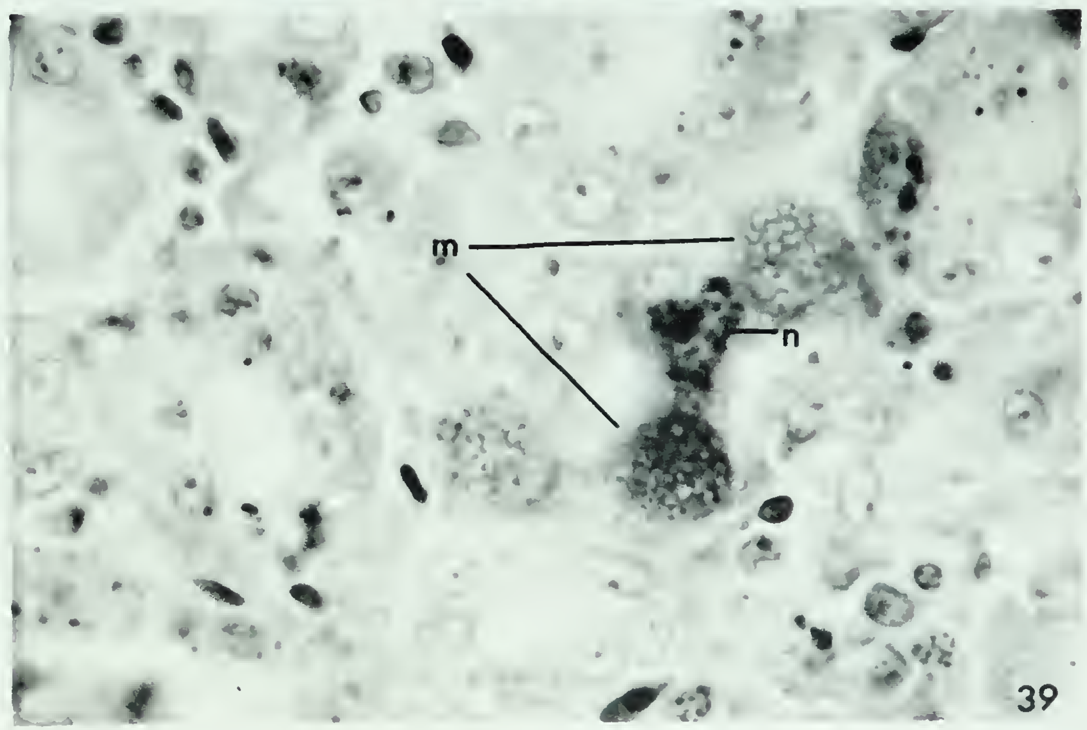


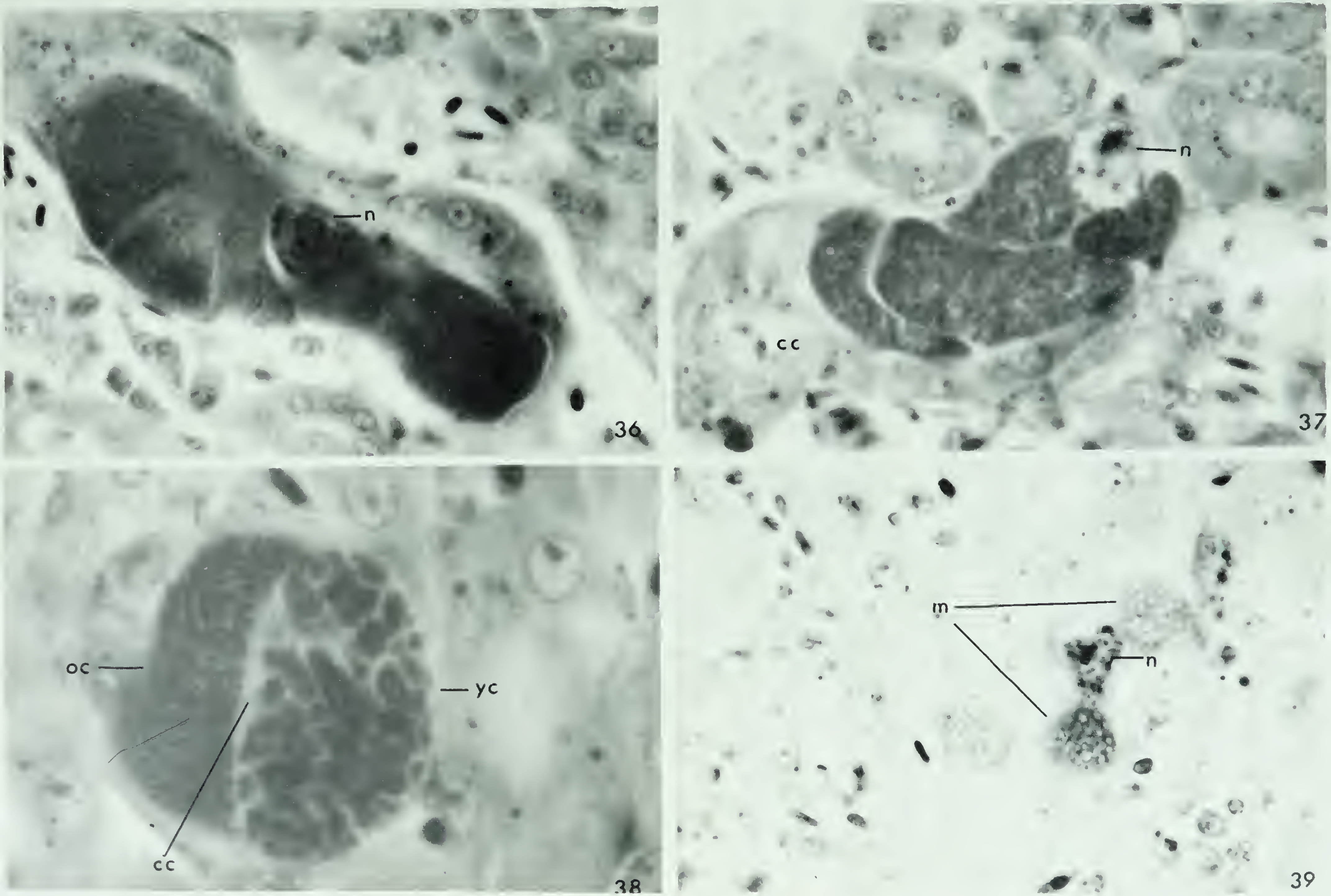
39

Figure 36. A relatively mature megaloschizont of Leucocytozoon. Note the nucleoid body (n). 2000X.

Figure 37. A relatively mature megaloschizont of Leucocytozoon, similar to Figure 36. Cytoplasmic clefts separating cytomeres can be clearly seen. Most sections do not show the nucleoid body. 2000X.

Figure 38. A megaloschizont showing two adjacent cytomeres in different stages of development. The older cytomere (oc) is at the left, the younger cytomere (yc) at the right. 3500X.

Figure 39. A ruptured megaloschizont of Leucocytozoon. Note the frothy appearance of the mature merozoites. 2000X.


n
36
n
cc
37
oc
yc
cc
38
m
n
39

sized clumps of yellow-brown granules of less than 1μ. The clumps were up to 25μ in greatest dimension. In sections stained using the Maximow technique the lipofuscin appeared green to greenish-blue. Seven grouse of various ages had unusually large amounts of this pigment in the liver. All of these birds had Leucocytozoon gametocytes in their tissues even though blood smears from three of the seven were negative. Lipofuscin also occurred in small amounts in some spleens, kidneys, and lungs. It occurred most often within phagocytic cells, but was also seen in many other cell types.

Hemosiderin, an iron-containing form of lipofuscin, was found in almost all livers and in six kidneys. It appears as very small granules, less than a micron in diameter. It was widely distributed in liver parenchyma, Kupffer cells, and free in interstitial areas and capillaries. It often occurred in intimate association with lipofuscin, and was most abundant in birds showing evidence of the schizogony of Leucocytozoon.

Melanin was also seen in four spleens and one liver.

Many of the spleens examined were enlarged and had a great proliferation of the white pulp. Some, in which the malpighian corpuscles were greatly enlarged and occupied most of the cross section, were very fragile and shattered when sectioned.

DISCUSSION

Variables affecting incidence of infection

Before attempting to draw conclusions in terms of the incidence and intensity of blood protozoan infections of Alberta grouse, an examination will be made of the variables affecting the results of this study. The results of studying blood smears will be examined first, since this technique is commonly used to survey various groups of animals for the presence of hematozoa.

Heart vs. peripheral blood

Fantham (1910a), in writing of *Leucocytozoon* infections in the red grouse, stated that "Gametocytes were more numerous in heart than in peripheral blood." In this study no differences were found. There were some cases in which parasites were seen in a bird's heart blood smear and not in that bird's peripheral blood smear, but just as frequently the reverse occurred. This was especially true in the case of trypanosomes which always appeared in very small numbers. While this would seem to indicate that examining both heart and peripheral blood smears might increase the probability of detecting low-grade infections, this would probably be accomplished as well by examining twice as many of one or the other type of smear.

The blood sampling technique used then should probably be determined by the requirements of the study. If, in doing a survey, one did not wish to injure the host, drawing peripheral blood from a wing vein or other superficial vessel would be preferred. In the case of obtaining blood smears from recently killed birds, drawing blood from the heart in most instances seems to yield better results.

Sex of the host

The data available from this study were too scant to determine

[illegible]

[illegible]

[illegible]

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the influence of the host's sex on incidence or intensity of infection. There were no apparent sexual differences. Borg (1953: 50) examined 603 grouse of three species and found "no demonstrable sexual disposition to infection with Leucocytozoon." In a study of blood parasites of yellow-billed magpies (Pica nuttalli), Clark (1966) did not find any differences between males and females.

Bennison and Coatney (1948) found that female chicks infected artificially with Plasmodium gallinaceum developed significantly greater parasitemias (62.3% of erythrocytes infected) than did male chicks (53% of erythrocytes infected).

Even if there were real differences involving the sex of the host, the temporal factors discussed below would tend to obscure these differences in all but extremely large samples or under laboratory conditions.

Age of the host

The age of a bird as a factor in susceptibility to infection is virtually unknown for the parasites concerned in this study. Experimental work, although numerous, has been confined entirely to the study of Leucocytozoon in ducklings (O'Roke, 1934; Huff, 1942; Fallis, Davies and Vickers, 1951; Chernin, 1952a, 1952b, 1952c; Cook, 1954; Cowan, 1955; Fallis, Anderson and Bennett, 1956; Briggs, 1960; Kocan and Clark, 1966a, 1966b; Desser, 1967). The small sample size and the influence of other variables mask the impact of age of the host on parasite load. For those studies in which large samples of both immature and adult birds were available, the results varied.

Dorney and Todd (1960) found that juvenile (10 to 12-month-old) ruffed grouse had a significantly lower incidence of infection with Leuco-

cytozoon and trypanosomes than did adult birds. Erickson *et al.* (1949) found that 72% of 114 immature and 67% of 49 adult ruffed grouse collected during a 7-year period were infected with *Leucocytozoon*. In a follow-up study, conducted during an ensuing 5-year period (Erickson, 1953), the results were similar; 71% of 115 immature and 68% of 38 adult grouse were infected. Sharp-tailed grouse (*Pedioecetes phasianellus campestris*) were examined in Michigan by Cowan and Peterle (1957). They found that 56% of 52 immature and 72% of 105 adult birds were infected with *Leucocytozoon*.

Trainer, Schildt, Hunt and Jahn (1962) found 13% of 52 adult wild-trapped mallards positive for *Leucocytozoon*. Among pen-raised mallards 91% of 83 immature and 67% of 24 adult birds were positive. Clark (1966) found no significant differences between immature and adult yellow-billed magpies, with the exception that *Plasmodium* was found more frequently in immatures than in adults. In English sparrows in Texas, Box (1966) found 39% of 128 immatures and 45% of 154 adults infected with *Plasmodium*. Of these same birds 24% of 128 immatures and 17% of 154 adults were infected with *Lankesterella*.

The work of Chernin (1952a, 1952b) on *L. simondi* clearly indicates that ducks of all ages are susceptible to this parasite. In addition, adult ducks are susceptible to relapse and to reinfection. Resistance to *L. simondi* was observed only with repeated infection, producing a high parasitemia (Fallis *et al.*, 1951). Manwell (1934) emphasized that once infected with a *Plasmodium* a bird apparently remains infected for life.

In light of the above it seemed evident that if any differences were to be expected between adult and immature birds, these differences should have favored a higher incidence of infection in adult birds, especially if the birds were examined in spring or summer (see seasonal

factors below). By this time infections in adult birds would have had the opportunity to relapse and the birds could have become infected during two or more summers. Why this expectation was seldom realized is a matter for speculation. Inadequate sampling may have played a part, but probably a minor one. The nature of the relapse phenomenon is probably important in that it affects the ease with which parasites may be detected. Chernin (1952b) found that parasitemia during the primary attack of L. simondi far exceeds that found during relapse. Borg (1953) reported that the intensity of Leucocytozoon parasitemias in adult grouse never reached the levels attained in some juveniles, and Wingstrand (1948) found gametocyte frequencies as high as 160 o/oo in juvenile crows, but never more than 5 o/oo in adults. These facts, plus the seasonal factors which will be discussed below, could account for many studies in which the prevalence of blood sporozoans in immature birds exceeded that of adults.

Seasonal factors

Dorney and Todd (1960) found that prevalence of Leucocytozoon in blood smears of Wisconsin ruffed grouse increased steadily from the first half of April to the last half of May. Parasitemia reached its height in May samples. Haemoproteus reached peak intensity and prevalence during the last half of May. Borg (1953) also reported peak prevalence of Leucocytozoon in Swedish grouse in May and June.

It is reported that the Haemoproteus of California quail (Herman and Bischoff, 1949) and of mourning doves (Farmer, 1962) do not seem to exhibit seasonal peaks of parasitemia, but instead show relapses at odd intervals. However, these birds were apparently kept in the laboratory for study and may not have been exposed to the stimulus or stimuli which trigger relapse in spring.

The peak parasitemia of *Leucocytozoon* and *Haemoproteus* in Dorney's study occurred just before the onset of hatching, and under experimental conditions Chernin (1952b) found that peak parasitemias of *L. simondi* coincided with the onset of laying. Thus, some physiological response to increasing day-length, or increased metabolic stresses associated with reproduction, may precipitate spring relapse of blood sporozoan infections.

Campbell (1954) reported a tendency for higher parasitemias of *Haemoproteus* and *Plasmodium* infections of 12 Gambel's quail (*Lophortyx g. gambeli*) to occur in September and October. All of these birds were young of the year. One adult female, kept with the young, also showed a slight rise in parasitemia in September. These results may have been due to repeated infection, as the birds were kept outdoors and vectors can be assumed to have been present. Studies on ruffed grouse hematozoa, based on material collected in summer and fall (Fallis, 1945; Erickson *et al.*, 1949; Dorney and Todd, 1960) as opposed to spring, resulted in significantly lower incidence of all blood parasites except *Plasmodium*.

Time of day

In this study the time at which blood smears were taken did not seem to affect the occurrence of protozoan blood parasites. No blood protozoans of grouse, except some species of *Plasmodium*, have been reported to show any periodicity (Wetmore, 1939). Coatney and West (1933) and Chernin (1952c) found no periodicity in *Haemoproteus columbae* or in *Leucocytozoon simondi* infections respectively.

The limited amount of material which is available for study precludes a detailed analysis of the above variables. Nevertheless, certain differences which were evident among the three groups of grouse will be discussed.

Table II shows that all of 13 adult ruffed grouse collected in central Alberta between May 1 and July 31, 1966, were blood-positive for Leucocytozoon. This is in marked contrast to only 6 of 13 immature birds collected in central Alberta, and only 15 of 29 ruffed grouse smears taken in the vicinity of the R. B. Miller Biological Station during the same period. At the same time 23 of 26 adult Franklin's grouse bled in the latter region were blood-positive for Leucocytozoon. Before concluding that there are differences between the two grouse species, and between the two areas, the following factors should be considered:

1. The apparently lower prevalence of Leucocytozoon among immature ruffed grouse was probably to be expected, as pointed out in the previous discussion.

2. The May thru July sample of central Alberta adult birds, although having 100% prevalence, is a small sample.

3. The prevalence among adult Franklin's grouse during the same months, although not 100%, is at 88%, still high. Most of the blood smears from these birds were very thick, and only a small area along the ends and margins of the smears could be examined for the presence of parasites. It is quite possible that some light parasitemias may have been missed.

4. The 52% prevalence in ruffed grouse from the R. B. Miller Biological Station does appear strikingly different from the 100% prevalence of the central Alberta birds, but as Table III shows, 23 of the 30 smears of Biological Station grouse were taken in May, whereas only 7 of the 13 central Alberta smears were made that month. The breeding season of central Alberta grouse preceded that of the Biological Station grouse by 2 to 4 weeks. Thus, the relapse of Leucocytozoon infection, if influenced by the host's reproductive cycle, might have been expected to occur from 2 to 4

weeks later at the Biological Station. This could account for much of the observed difference in prevalence between the two areas.

5. The vector or vectors of Leucocytozoon in either area is not known. All that can be said of the likelihood of infection in the two areas is that the area around the R. B. Miller Biological Station provides at least as good a habitat for Simuliids as does most of the central Alberta areas from which grouse were collected. However, from the brief discussion of the flight range of Simuliids presented by Fallis (1964), it can be seen that the absence of black fly breeding areas, in the immediate vicinity where a bird was collected, does not necessarily mean that this vector is not present.

The incidence of Haemoproteus in blood smears of the three groups during the May to August period is the reverse of that of Leucocytozoon. Twenty-eight of 40 Franklin's grouse were blood-positive for Haemoproteus, as compared to 8 of 30 ruffed grouse from near the R. B. Miller Biological Station, and only 3 of 13 ruffed grouse from central Alberta. The relatively low (27%) prevalence in Biological Station ruffed grouse may again reflect the fact that the vast majority of these smears were made much earlier in the year than were the Franklin's grouse smears, since Haemoproteus also seems to increase in the peripheral circulation later in the breeding season (Dorney and Todd, 1960). The seemingly greater prevalence of Haemoproteus at the Biological Station area as compared to central Alberta may reflect a greater vector abundance at that area. Because the vectors of Haemoproteus are even less well understood than those of Leucocytozoon, this is a difficult area for speculation. Hippoboscids, known vectors for several species of Haemoproteus (O'Roke, 1930; Huff, 1932; and Baker, 1966b), were not found on any of the birds in this study, although several species have been reported

from Alberta (Appendix I). Midges of the family Ceratopogonidae have been shown to transmit _Haemoproteus_ to spruce grouse and ducks in Ontario (Fallis and Wood, 1957; Bennett and Fallis, 1960). These midges may well be the vectors of _Haemoproteus_ in Alberta grouse. Because of their minute size they are easily overlooked, and have not been well studied in Alberta. Undoubtedly, many more species are present than are indicated in the appendix.

Trypanosomes were also more abundant in birds from the area of the R. B. Miller Biological Station. As with _Haemoproteus_ this may reflect a difference in vector abundance. Baker (1956) found a hippoboscid to be the vector of _T. avium_ in English birds. However, Bennett and Fallis (1960) found that transmission of avian trypanosomes in Ontario could be accomplished by black flies, biting midges, mosquitoes, and hippoboscids. They also found that _Trypanosoma_ and _Leucocytozoon_ occurred together more frequently than any other two parasites, suggesting a common vector. Stabler, Kitzmiller and Weeden (1967) found _Trypanosoma_ in 23 Alaskan rock ptarmigan, 19 of which also were infected by _Leucocytozoon_.

No seasonal trends in trypanosome incidence were apparent from the scant data available. Many infections may have been missed because so few organisms are usually present in the blood (Borg (1953) never found more than 5 to 10 trypanosomes per slide). Diamond and Herman (1954) have shown that with the Canada goose (_Branta canadensis_ L.) the detection of trypanosome infections could be greatly increased by culturing bone marrow samples from the birds being studied.

Comparison with other studies of grouse hematozoa

The early British studies concerning blood parasites of the red grouse (Seligmann and Sambon, 1907; Sambon, 1908; Fantham, 1910a) and early

North American studies on ruffed grouse, sharp-tailed grouse, and willow ptarmigan (Stafseth and Kotlan, 1925; Allen, 1924; Allen and Levine, 1935; Clarke, 1935; Wetmore, 1939) presented no data on incidence or intensity of infection. These early investigators were, for the most part, content to name, and occasionally to describe, the hematozoa which they found.

Later studies of North American tetraonids by Fallis (1946); Fowle (1946); Erickson *et al.* (1949); Adams and Bendell (1953); Erickson (1955); Cowan and Peterle (1957); Bennett and Fallis (1960); Dorney and Todd (1960); Holmes and Boag (1965); Stabler, Kitzmiller, Ellison and Holt (1967); Stabler, Kitzmiller and Weeden (1967); and of European tetraonids by Oliger (1940) and Borg (1953) have reported varying amounts of data concerning incidence and intensity of infection. Table VI summarizes the results of these studies.

The incidences of infection found in the present study can be compared only roughly with those shown in Table VI. Clarke's (1935) report of 100% incidence of *Leucocytozoon* makes no mention of the number of birds examined. Oliger's (1940) data on the hazel hen (*Tetrastes bonasia*) provides no data on the number of birds examined, and incidence is given on a monthly basis and ranges from 31 to 100%. Erickson's (1953) report spans 11 years, during which the incidence of *Leucocytozoon* infection varied from 55 to 100%.

Very few objective reports of intensity of infection have been provided in the literature. When a measure of intensity was made it was usually presented as the number of gametocytes seen per unit of time searched, or the number seen per oil immersion field. These data are virtually useless for comparative purposes because they are influenced by the efficiency of the investigators, differing densities of blood cells in different areas

Table VI. Protozoan blood parasites reported from the Tetraonidae.

Author	Date	Location	Host	Incidence (in % if known) L	H	T	Remarks
Adams & Bendell	1953	British Columbia	blue grouse	87 46	92 80	76 17	adult birds juvenile birds
Allen & Levine	1935	Northwest Territories	willow ptarmigan	+			
Bennett & Fallis	1960	Ontario	ruffed grouse	73	8	24	75 birds examined
Bennett & Fallis	1960	Ontario	spruce grouse	50	33	25	12 birds examined
Borg	1953	Sweden	hazel hen	63			41 birds examined
Borg	1953	Sweden	black grouse	53			104 birds examined
Borg	1953	Sweden	capercaillie	72			215 birds examined; *Plasmodium* in 1 bird
Bowers & Tannar	1950	Pennsylvania	ruffed grouse	+	+	+	
Clarke	1935	Ontario	ruffed grouse	100		+	
Clarke	1935	Ontario	spruce grouse	+		+	
Clarke	1938	Ontario	ruffed grouse		"quite heavy".		
Cowan & Peterle	1957	Michigan	sharp-tailed grouse	66*			*66% of smears examined; multiple smears made from many birds; mostly winter-trapped birds
Dorney & Todd	1960	Wisconsin	ruffed grouse	86	58	32	
Erickson	1953	Minnesota	ruffed grouse	71		1	316 birds examined
Erickson *et al*.	1949	Minnesota	ruffed grouse	71	0	+	163 birds examined

Table VI continued.

Author	Date	Location	Host	Incidence (in % if known) L	H	T	Remarks
Fallis	1945	Ontario	ruffed grouse	63	16	6	106 birds examined; 7% with Plasmodium
Fantham	1910	Scotland	red grouse	+	+		
Fowle	1946	British Columbia	blue grouse	18	52	5	44 birds examined
Holmes & Boag	1965	Alberta	ruffed grouse	+			56 birds examined
Holmes & Boag	1965	Alberta	blue grouse	+	+	+	85 birds examined
Holmes & Boag	1965	Alberta	spruce grouse	+			20 birds examined
Laird	1961	Northwest Territories	rock ptarmigan	0	0	0	9 birds examined
Oliger	1940	Russia	hazel hen	31 to 100			depending on month examined
Sambon	1908	Europe	capercaillie	+			
Seligmann & Sambon	1907	Scotland	red grouse	+			
Stabler *et al*.	1967	Alaska	rock ptarmigan	89	0	14	summer collections - 162 birds examined
Stabler *et al*.	1967	Alaska	spruce grouse	82	62	41	summer-fall collections - 221 birds examined; also 1 bird with Hepatozoon and 2% with Plasmodium
Wetmore	1939	North Dakota	sharp-tailed grouse				Plasmodium

L = Leucocytozoon   H = Haemoproteus   T = Trypanosoma

of a smear and between different smears, and even the different fields of view of various microscopes.

Cowan and Peterle (1957) and Dorney and Todd (1960) developed indices of infection intensity for Leucocytozoon gametocytes based on the number of gametocytes seen per oil immersion field; however, they used different indices. Only Adams and Bendell (1953) and Borg (1953) have presented figures on the numbers of Leucocytozoon gametocytes seen per 1,000 erythrocytes, although some other investigators have used this technique in reporting Haemoproteus infections.

Borg (1953: 45) defined an "acute" stage of Leucocytozoon infection in grouse as the "stage when gametocyte frequency exceeds some 1 o/oo of the erythrocytes." In accordance with its use in most of the literature on avian hematozoa, "acute" refers to parasitological rather than to clinical data.

In Borg's study parasitemias of greater than 1 o/oo were limited to juveniles of all three species of grouse examined. A summary of the parasitemias reported by Borg follows:

8 of 97 infected juvenile capercaillie had >.5 o/oo
(from 1 to 8 o/oo)

146 infected adult capercaillie never had >.5 o/oo
(usually <.1 o/oo)

2 of 34 infected juvenile black grouse had >.5 o/oo
(1 o/oo and 3 o/oo)

47 infected adult black grouse never had >.5 o/oo
(usually <.1 o/oo)

4 juveniles of 22 infected hazel hens (all ages) had
>.5 o/oo (2 to 15 o/oo)

In contrast to these figures it is of interest to note that the two "acute" infections of Leucocytozoon found in the present study were both in adult birds--2 o/oo in a ruffed grouse and 1 o/oo in a Franklin's grouse. Adams and Bendell (1953) reported Leucocytozoon parasitemias of 1 o/oo or 2 o/oo in sooty grouse (Dendragapus obscurus fuliginosus) on Vancouver Island.

The reported incidence of Haemoproteus in grouse ranges from 8% in Ontario ruffed grouse (Bennett and Fallis, 1960) to 92% in adult sooty grouse (Adams and Bendell, 1953). It can be seen from Table V that incidence of infection in spruce grouse has usually been greater than in ruffed grouse (except for Dorney and Todd, 1960). As previously discussed this may be a reflection of differential vector abundance in the respective habitats of these birds.

Reported incidence of trypanosomes in grouse ranges from 1.2% in Minnesota ruffed grouse (Erickson et al., 1949) to 76% in adult sooty grouse (Adams and Bendell, 1953).

Plasmodium has been reported twice from ruffed grouse (Fallis, 1945; Dorney and Todd, 1960), once from sharp-tailed grouse (Wetmore, 1939), once from spruce grouse (Stabler, Kitzmiller, Ellison and Holt, 1967), and once from the hazel hen (Borg, 1953). In addition, unpublished observations have been made of Plasmodium in Alberta sharp-tailed grouse. Unless segmenting stages are seen in the blood Plasmodium and Haemoproteus are easily confused. If a heavy infection of Plasmodium occurred in a bird which was not also infected with Haemoproteus, it would probably have been noticed, but if mixed infections occurred it might be overlooked. In the present study a determined effort was made to locate Plasmodium, but this parasite could not be found with certainty. In fact, only a very few gametocytes

looked suspiciously like Plasmodium. Presumably, the mosquito vector of this parasite was absent from the areas studied, although mammalophilic mosquitoes were present in abundance. Herman, Knisley and Snyder (1966) have discussed the desirability of subinoculation of blood into an alternate host to demonstrate subpatent Plasmodium infections. Unfortunately, this technique was beyond the scope of the present study.

The blood parasites of other gallinaceous birds have been studied in North America, especially turkeys (Skidmore, 1932; Johnson, Underhill, Cox and Threlkeld, 1938; West and Star, 1940; Banks, 1943; Hinshaw and McNeil, 1943; Byrd, 1959) and quail (O'Roke, 1930; Herman and Glading, 1942; Campbell and Lee, 1953).

Asexual stages

Of the studies of grouse hematozoa presented in Table VI, only Fantham (1910b), Clarke (1935), and Borg (1953) attempted to find asexual stages of the parasites in tissues other than the blood. Indeed, with the exception of the genus Plasmodium, which has been extensively studied (see review by Porter and Huff, 1940), Leucocytozoon simondi (Ivanic, 1937; Huff, 1942; Cowan, 1955; Cowan and Peterle, 1957; Desser, 1967), L. sakharoffi in Swedish crows (Wingstrand, 1947, 1948), and L. berestneffi in the yellow-billed magpie (Clark, 1965), no detailed studies exist of the asexual stages of avian blood parasites, although some investigators (Clarke, 1938; Manwell, 1951; Borg, 1953; Baker, 1966a) claim to have seen a few exoerythrocytic stages in some of the birds which they examined.

Assessing the extent of asexual multiplication of hematozoa in the avian host, or the incidence of these stages in a population, is fraught with even more difficulty than that involved in determining incidence and intensity by examination of blood smears. In addition to all of the

variables mentioned in the preceding discussion, there is the added problem of selection of specific organs. When a large organ such as liver, kidney, or brain is examined, there is the question of what part and how much of the organ to examine. Negative results from the histologic examination of a few microns of tissue from one of these organs cannot help but leave one wondering if the results obtained from a different piece of the tissue of that organ might not have been positive.

Fantham (1910b) was the first to report on schizogony of Leucocytozoon in grouse. He described "schizonts" from spleen impression smears of red grouse which were blood-positive for Leucocytozoon. From his description and illustrations it appears that these "schizonts" were not intracellular. His measurements put them in the size range of Leucocytozoon gametocytes. They do not resemble any subsequently reported schizonts of any species of Leucocytozoon. For these reasons I believe that they must be discounted as being schizonts of Leucocytozoon. They may have been schizonts of some species of Plasmodium or Lankesterella, although Fantham stated that the birds exhibiting these schizonts were not infected by any hematozoon other than L. lovati. However, since subinoculation was not made, Plasmodium cannot be ruled out (see Herman et al., 1966).

Clarke (1938: 146) examined sections of "various tissues" of ruffed grouse and found schizonts of L. bonasae in "all grouse old enough to show infection and at all seasons." However, Clarke makes no mention of how many grouse were examined, does not state in which tissues schizonts were found, and it is impossible to tell from the one photograph accompanying the text just what the schizonts looked like, and their appearance is nowhere described.

Borg (1953), the only other author to have seen asexual forms of *Leucocytozoon* in grouse, found some parasites resembling Huff's (1942) hepatic schizonts of *L. simondi* in the livers of some juvenile grouse which had *Leucocytozoon*-positive blood smears. The asexual stages of *Leucocytozoon* found in ruffed grouse from this study are illustrated in Figures 28 to 39. The earliest stages found (Figures 28 to 31) are similar to those described by Ivanic (1937) and Huff (1942) for *L. simondi*, by Clark (1965) for *L. berestneffi*, and by Wingstrand (1948) for *L. sakharoffi*.

The intensity of staining of these small hepatic schizonts by the Azure II stain was quite variable. All attempts to use iron hematoxylin staining to better elucidate their morphology failed. This may have been due to loss of affinity for basic stains which sometimes occurs in tissues fixed with Zenker-formol fixatives (Bowling, 1967). The Feulgen reaction for DNA was weak or nonexistent in these small schizonts. Wingstrand (1948) found this to be true of schizonts of *L. sakharoffi* until the schizonts were quite mature, although Porter and Huff (1940) found that tissue schizonts of *P. elongatum* were Feulgen-positive, and Clark (1965) also reports positive Feulgen reactions with early schizonts. Techniques of fixation and hydrolysis are known to have profound effects on the results of Feulgen staining, and this may be part of the problem, although it was possible to achieve good staining of the host tissue.

As shown in Table IV, small schizonts were found in liver sections in 27 of 38 ruffed grouse, and may have been present in at least two others. Similar small, intracellular schizonts were also seen in the kidneys of three birds (Figure 34).

The large schizonts (Figures 35 to 39) seen in the kidneys of two grouse were not found in any other organ. I believe these to be

homologous to the megaloschizonts of L. simondi originally described by Ivanic (1937), and subsequently studied by Huff (1942), Cowan (1955), and Desser (1967), and of L. sakharoffi by Wingstrand (1948). They in no way resemble the schizonts found in the kidneys of the purple grackle (Quiscalus quiscala quiscala) by Manwell (1951). Ivanic's illustrations of large schizonts in the kidney of the domestic goose most resemble those found in the present study.

It cannot be stated at this time whether or not the distribution of megaloschizonts in grouse is similar to that found with other species of Leucocytozoon. In L. simondi infections megaloschizonts have been reported from most organs of the host. For example, Cowan (1955) found megaloschizonts in the spleen, heart, bursa of Fabricius, small intestine, adrenals, brain, and kidney of Pekin ducklings. In this study large schizonts were found only in the kidney. While it cannot be stated with any degree of certainty, on the basis of two birds, that large schizonts occur only in the kidney, the fact that none of the other organs examined from these two birds, or from any of the other 38 birds, contained large schizonts tends to suggest that this might be the case, at least as concerns the organs which were examined in this study. Since experimental work with L. simondi has shown that the life span of megaloschizonts is a matter of 3 or at most 4 days, it is easy to understand the difficulty of finding these stages in naturally-infected wild birds. It is not so easy to understand how they could be found in abundance in one organ and not in any of the others from the same bird, if indeed they occur in other organs. There is the possibility that megaloschizonts were present in other organs but, due to a spotty or localized distribution, they did not occur in the piece of tissue sampled. Another possibility is that the distribution of

schizonts during relapse (which was probably occurring in the grouse that had megaloschizonts, since they were adult birds collected in the spring) is different than the distribution in juvenile birds which had had no previous experience with the parasite, as is the case with all experimental studies reported in the literature.

In most respects the large schizonts found in this study compare favorably with those reported from L. simondi infections; however, a comparison of their development to that of other species cannot be made, as all those found in the grouse were relatively mature. The central body (Figures 36 to 38) described by Huff (1942) and Cowan (1955), and which I have called the nucleoid body, usually was not encountered. This was probably because of the mature nature of the schizonts. However, the elongate form assumed by schizonts growing in the kidney, unlike the nearly spherical form of those found in the spleen, reduced the possibility of a section passing through the central body even when it was present, and reduced the chance of encountering it. Cytoplasmic clefts (Figure 37) and the development side by side of different aged cytomeres (Figure 38), described by Huff and Cowan, did occur in the grouse material.

The occurrence of large schizonts is not known to be a universal phenomenon of Leucocytozoon infections. Clark (1965) examined organs (spleen, bursa of Fabricius, heart, small intestine, large intestine, brain, kidney, lung, and liver) of 72 magpies infected with L. berestneffi. No megaloschizonts were found although small hepatic, renal, and splenic schizonts did occur. Borg (1953) was unable to find any large schizonts of Leucocytozoon in the tissues of capercaillie, black grouse, or hazel grouse.

Pathology of Leucocytozoon in grouse

As stated in the introduction one of the aims of this study was to determine the effects of blood parasites on the host, as evidenced in histologic sections of the organs studied. With the possible exception of O'Roke (1930) those who have studied Trypanosoma and Haemoproteus in birds have found no evidence of pathogenicity by these hematozoa. However, at least one species of Leucocytozoon (L. simondi) is known to cause extensive mortality among infected ducklings and goslings (Wickware, 1915; Knuth and Magdeburg, 1922, 1924; O'Roke, 1934). Another species, L. smithi, has been suspected of causing severe losses among flocks of domestic turkeys (Smith, 1895; Laveran and Lucet, 1905; Skidmore, 1932; Johnson et al., 1938; Hinshaw and McNeil, 1943; Savage and Isa, 1945; West and Star, 1940). Evidence for the pathogenicity of L. smithi is very tenuous however (see Borg, 1953).

Clarke (1934, 1935) was the first to suggest that the Leucocytozoon found in grouse caused extensive mortality in these birds, over 80% of the juvenile grouse succumbing to the parasite. Subsequent studies by other investigators have failed to demonstrate that this is the case.

If Leucocytozoon were a factor in the mortality of grouse there are three obvious mechanisms by which the death of the host might occur.

Some protozoan parasites can cause death of their host by sheer weight of numbers, destroying tissues, competing for nutrients, or completely occluding blood vessels. This does not seem to be the case with any avian hematozoon infections, except that in the genus Plasmodium surface alterations may occur in infected erythrocytes such that they no longer flow smoothly through blood vessels (Knisely, Stratman-Thomas, Eliot and Bloch, 1964).

Production of toxins upon the destruction of large numbers of

parasites sometimes occurs in sporozoan infections involving Sarcosporidia, Toxoplasma and Eimeria, but there is no evidence of this phenomenon in any of the genera found in this study.

Destruction of erythrocytes and subsequent debilitating anemia could be a third mechanism. Although destruction of blood cells does undoubtedly occur the parasitemia reached in grouse could not directly account for the destruction of enough blood cells to cause any great hardship to the host. Indeed, Haemoproteus gametocytes, which occur much more abundantly, cause the destruction of many more cells. Nevertheless, in studies of L. simondi infections severe anemias have been reported near the peak of patency (Fallis et al., 1951). The cause of these anemias has yet to be discovered. They are too severe to be accounted for by the destruction or phagocytosis of infected cells (Kocan and Clark, 1966b). Anemias of even greater magnitude which were caused experimentally in ducklings did not produce any permanent debility (Kocan, personal communication). Autoimmune reactions whereby the host produces antibody against its own red blood cells could occur, but no good evidence for this exists. Extensive hemolysis can be fatal by causing renal failure because of reduction of the blood supply to the renal cortex, as occurs with Plasmodium falciparum infections in man (Giglioli, 1962).

MacCallum (1940) states that with respect to human Plasmodium infections the harmful effects are dependent on the asexual cycle alone. Tissue stages of Leucocytozoon have been studied from the standpoint of pathology by Cowan (1957) and Newberne (1955). The extensive invasion and phagocytosis of many megaloschizonts, as described by Cowan and Newberne for L. simondi, was not observed in the present study. In fact, there was no histologic evidence of host reaction to the presence

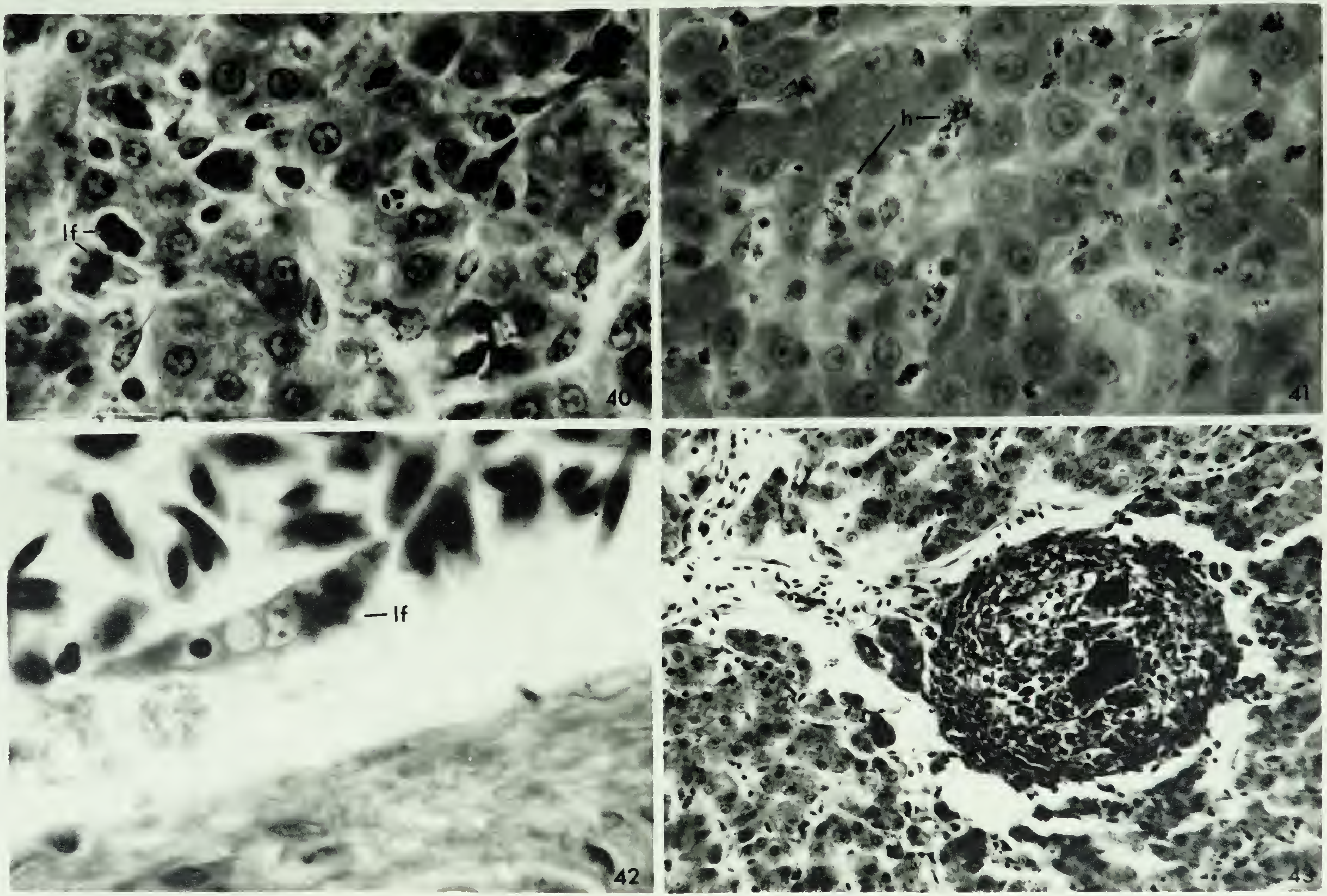
of the parasites in the kidney. Cowan described the inflammatory reaction to megaloschizonts from the spleen of infected ducks. Newberne found this reaction only in the brain and lungs. The liver of one of the birds which had large schizonts in its kidney, as well as the liver of another grouse, had necrotic areas containing much cellular debris and granulocytes. There was marked lymphocytic infiltration in surrounding areas. Giant cells were often present (Figure 43). These lesions appear to be similar to those seen by Newberne in the livers of infected ducklings. He attributed this liver damage to anoxia caused by the anemia accompanying the infection. Erythrophagocytosis was observed in many liver sections. Hemosiderosis, found in grouse by Borg (1953) and in ducklings by Newberne, was also seen in the livers and spleens of the moderate to heavily-infected grouse examined in this study, suggesting that the destruction of large numbers of erythrocytes was indeed occurring. In most infected birds, especially those that had many hepatic schizonts, lipofuscin (ceroid) was found. In livers in which schizonts were abundant lipofuscin occurred within the same cells that harbored schizonts. The presence of this pigment in abnormal amounts is usually indicative of pathological conditions in most animals (Trautwein, 1952). The processes involved in its elaboration are not well understood, but in the present study it is probably indicative of liver damage. This pigment has not been previously reported as being associated with *Leucocytozoon* infections, and its presence in grouse needs further study to clarify the amounts normally present in healthy birds and its relationship to disease. The granules of this pigment may have been mistaken for schizonts by some of the earlier investigators.

Figure 40. A large amount of lipofuscin (lf) in the liver of a ruffed grouse. 2000X.

Figure 41. Hemosiderin (h) in the liver of a ruffed grouse. Perl's Prussian blue reaction. 2000X.

Figure 42. A macrophage containing a large clump of lipofuscin pigment. This was a common sight in sections of most organs. 3500X.

Figure 43. A necrotic area in a liver section. These well-defined necrotic areas were scattered throughout the section. The large, dark-staining bodies in the center of the necrosis are giant cells. 500X.



lf
40
h
41
lf
42

SUMMARY

In attempting to answer the question, "Do protozoan blood parasites cause population fluctuations in grouse?", one must show that these parasites are capable of causing their host's death. This is difficult to demonstrate outside of a laboratory situation. In the present study it was hoped that pathological changes in the vital organs of grouse might implicate blood parasites as a mortality factor.

Certainly none of the grouse examined exhibited morbidity to the extent that it would seem to have been moribund at the time it was collected. Borg (1953) did not find one unquestionable case of death caused by *Leucocytozoon* in 604 grouse. However, this by no means precludes the possibility that serious mortality can be caused by the parasite in question. Even in the case of *L. simondi*, where mortality is well documented, this mortality is confined to young ducklings and goslings. In the case of ruffed grouse this age group of 3 to 5-week-old birds is perhaps the most difficult to collect, and material is sadly lacking in this and other studies (Erickson, 1953). At this age the chicks have been exposed to vectors, if present, and the parasite has had time to reproduce asexually in the bird. More pathological studies are needed of this age group, along with information on vector abundance and grouse population densities, to obtain a picture of the epizootiology of blood protozoan infections.

This study has contributed to the knowledge of the distribution of protozoan blood parasites in a little-studied area. The apparent absence of *Plasmodium* sp. from Alberta's forest grouse needs to be further investigated. As with *Leucocytozoon* and *Haemoproteus*, the first step needed is to determine the vector or vectors of the parasite.

Determinations as to species of the parasites must await cross-

transmission studies, as well as studies of the sporogonic cycle in the dipteran host(s).

The tissue stages of Leucocytozoon in grouse have been described in detail for the first time. Megaloschizonts have not previously been observed in grouse, having only been described from ducks, geese, and crows. The discovery of this stage has great implications in regard to the potential pathogenicity of Leucocytozoon in grouse. A great deal more work needs to be done on these stages, especially to determine the extent of organ involvement.

The presence of large amounts of lipofuscin seems not to have been observed in other hosts infected by Leucocytozoon, and needs to be further investigated to determine its relationship to tissue damage caused by the parasites.

The combined data available from blood and tissue examination (Table V) indicate that the actual prevalence of Leucocytozoon is even greater than would be found by blood smear study alone—100% as opposed to 60. It should be noted that surveys done during the fall hunting season would be of little value.

The implications of the high prevalence of Leucocytozoon in the population are clear. Given a high density of infected breeding birds, along with conditions conducive to vector abundance, it is entirely conceivable that newly hatched chicks would be heavily infected with sporozoites. With the tremendous potential of each megaloschizont to produce hundreds of thousands of merozoites, the host's defense mechanisms might easily be overwhelmed. At the height of patency we can expect that the bird is placed in greater jeopardy from other mortality factors.

Haemoproteus needs even more study than Leucocytozoon. The exo-

erythrocytic stages in grouse are still unknown. Franklin's grouse, at least in the area of the R. B. Miller Biological Station, would seem to be an ideal subject for study because of the apparently high prevalence of Haemoproteus in the population, and the relative ease with which the birds are captured--once they are located.

The incidence and intensity of trypanosome infection in this and in other studies seem to be so low that they cannot even be considered suspect as a serious mortality factor.

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APPENDIX I

A list of Simuliidae, Ceratopogonidae, and Hippoboscidae collected in Alberta

The following list is taken from Abdelnur (1966)[1] and from the collections of the Strickland Memorial Museum of the Department of Entomology, University of Alberta. Known vectors of avian blood protozoa are indicated in the footnotes.

SIMULIIDAE

Simulium	(Simulium)	decorum Walker, 1848
		hunteri Malloch, 1914
		tuberosum Lundstroem, 1911
		luggeri Nicholson and Mickel, 1950
		venustum Say, 1823[2]
		verecundum Stone and Jamnback, 1955
		meridionale Riley, 1886
		malyshevi Dorogostajakij, Rubtzov and Vlasenko, 1935
		piperi Dyar and Shannon, 1927
	(Byssodon)	rugglesi Nicholson and Mickel, 1950[3]
		transiens Rubtzov, 1949
	(Gnus)	arcticum Malloch, 1914
		corbis Twinn, 1936
	(Psilopelmia)	griseum Coquillett, 1898
		bivittatum Malloch, 1914

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[1] The biology of some black flies (Diptera: Simuliidae) of Alberta. Ph.D. Thesis, Department of Entomology, University of Alberta.

[2] L. simondi, Fallis et al., 1951

[3] L. simondi, Fallis et al., 1956

	(*Psilozia*)	*vittatum* Zetterstedt, 1838
	(*Eusimulium*)	*aureum* Fries, 1824[3]
		latipes (Meigen), 1804
		pugetense Dyar and Shannon, 1927
	(*Hagenomia*)	*pictipes* Hagen, 1880
Cnephia	(*Cnephia*)	*dacotensis* Dyar and Shannon, 1927
		emergens Stone, 1952
		saskatchewana Shewell and Fredeen, 1958
	(*Stegopterna*)	*mutata* (Malloch), 1914
	(*Cnetha*)	*saileri* Stone, 1952
Prosimulium	(*Prosimulium*)	*fulvum* (Coquillett), 1902
		pleurale Malloch, 1914
		travisi Stone, 1952
		decemarticulatum (Twinn), 1936[4]
		onychodactylum Dyar and Shannon, 1927

Twinnia biclavata Stone and Jamnback, 1955

CERATOPOGONIDAE

Bezzia sp.

Ceratopogon cockerelli

Culicoides obsoletus

Dasyhelea cincta

Forcipomyia cilipes

Forcipomyia specularis

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[3] *L. mirandae*, *L. fringillinarum* and *L. bonasae*, Fallis and Bennett, 1962

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HIPPOBOSCIDAE

Lynchia albipennis

Linchia nigra

Ornithoetona erythrocephala

Ornithomyia fringillina

APPENDIX II

Which blood cells are infected by *Leucocytozoon* gametocytes?

This question has caused a great deal of controversy ever since the parasite was first discovered. In his book on blood parasites Danilewsky (1889) suggested that the membrane enveloping *Polimitus major* might be the remains of a degenerated leucocyte. In a second paper on the subject Danilewsky (1890) proposed that the "cytocapsule" surrounding the parasite was actually formed by a hemocytoblast (erythroblast) and not by a leucocyte. Illustrations in the 1890 paper clearly indicate that the parasite being discussed is actually *Leucocytozoon*.

Sakharoff (1893) found *Leucocytozoon* in the blood of the raven (*Corvus corax*), the rook (*Trypanocorax frugilegus*), and the magpie (*Pica pica*) and stated that the parasitized cells were leucocytes. Laveran (1902) described a leucocytozoon from the blood of the great tit (*Parus major*) and claimed to have ascertained that the parasites were enclosed within greatly altered erythrocytes, because the staining affinities of the protoplasm of the enveloping cells more nearly resembled those of erythrocytes. Berestneff (1904) found leucocytozoa in an owl, a raven, and a magpie. In all cases he considered the parasites to be within leucocytes. Laveran and Lucet (1905) found a *Leucocytozoon* (described in 1895 by Theobald Smith) in young turkeys. Contrary to Laveran's previous statement concerning the *Leucocytozoon* of the great tit, they suggested that the elements enclosing the parasite were leucocytes. Sambon (1908) agreed with Danilewsky (1890) and regarded the host cell to be a greatly enlarged and altered erythroblast.

O'Roke (1934) considered the gametocytes of *L. simondi* to develop in erythrocytes, although he reports finding young gametocytes

in all types of leucocytes as well. Huff (1942) stated that the only cells in which he could find closely spaced stages of growth from young to mature gametocytes were lymphocytes. Fallis *et al.* (1951) reported finding gametocytes in erythrocytes and lymphocytes.

Cook (1954) studied the host cell in *L. simondi* infections. By applying the benzidine peroxide staining method of Ralph (1941) she claimed to have demonstrated that the majority of young gametocytes were found in cells containing hemoglobin. Clark (1965) also employed this technique in studying gametocyte development of *L. berestneffi* in the yellow-billed magpie (*Pica nuttalli*). He found no evidence of hemoglobin in any of the parasitized cells. Desser (1967) claims to find the very young gametocytes of *L. simondi* in erythrocytes.

In the present study all of the invaded cells appear to be either lymphocytes or erythroblasts. Extensive searching of slides on which very young gametocytes appeared failed to reveal any parasitized erythrocytes.

Cook's results, regarding the hemoglobin content of parasitized cells, may be questionable. From her description of the same cells which she concurrently stained with Giemsa, it is evident that she was experiencing some staining problems, although she was apparently unaware of them. Of course, Clark's (1965) failure to duplicate her results with *L. berestneffi* could be due to species-specific differences.

Other hematozoa known to parasitize erythrocytes (i.e. *Plasmodium* and *Haemoproteus*) produce a pigment (hemozoin) as a breakdown product of hemoglobin. However, it has been demonstrated that in *Babesia rodhaini* infections phagotrophy of erythrocytic cytoplasm occurs without the production of a residual pigment (Rudzinska and Trager, 1962).

From the above discussion one could conclude that either (1)

Leucocytozoon gametocytes do not occur in erythrocytes, or (2) they are species-specific with each species invading one or more types of host cell. In the latter case those that invade erythrocytes either (1) do not utilize the host cell's cytoplasm as food, or (2) if they do, their metabolism differs from that of *Plasmodium* and *Haemoproteus*.